Chemical Modification of Cellulose Esters for Oral Drug Delivery

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ABSTRACT

Polymer functional groups have critical impacts upon physical, chemical and mechanical properties, and thus affect the specific applications of the polymer. Functionalization of cellulose esters and ethers has been under extensive investigation for applications including drug delivery, cosmetics, food ingredients, and automobile coating.

In oral delivery of poorly water-soluble drugs, amorphous solid dispersion (ASD) formulations have been used, prepared by forming miscible blends of polymers and drugs to inhibit crystallization and enhance bioavailability of the drug. The Edgar and Taylor groups have revealed that some cellulose ω-carboxyalkanoates were highly effective as ASD polymers, with the pendant carboxylic acid groups providing both specific polymer-drug interactions and pH-triggered release through swelling of the ionized polymer matrix. While a variety of functional groups such as hydroxyl and amide groups are also of interest, cellulose functionalization has relied heavily on classical methods such as esterification and etherification for appending functional groups. These methods, although they have been very useful, are limited in two respects. First, they typically employ harsh reaction conditions. Secondly, each synthetic pathway is only applicable for one or a narrow group of functionalities due to restrictions imposed by the required reaction conditions.

To this end, there is a great impetus to identify novel reactions in cellulose modification that are mild, efficient and ideally modular. In the initial effort to design and synthesize cellulose esters for oral drug delivery, we developed several new methods in cellulose functionalization, which can overcome drawbacks of conventional synthetic pathways, provide novel cellulose derivatives that are otherwise inaccessible, and present a platform for structure-property relationship study.

Cellulose ω-hydroxyalkanoates were previously difficult to access as the hydroxyl groups, if not protected, react with carboxylic acid/carbonyl during a typical esterification reaction or ring opening of lactones, producing cellulose-*g*-polyester and homopolyester. We demonstrated the viability of chemoselective olefin hydroboration-oxidation in the synthesis of cellulose ω-hydroxyesters in the presence of ester groups. Cellulose esters with terminally

olefinic side chains were transformed to the target products by two-step, one-pot hydroboration-oxidation reactions, using 9-borabicyclo[3.3.1]nonane (9-BBN) as hydroboration agent, followed by oxidizing the organoborane intermediate to a primary alcohol using mildly alkaline H_2O_2. The use of 9-BBN as hydroboration agent and sodium acetate as base catalyst in oxidation successfully avoided cleavage of ester linkages by borane reduction and base catalyzed hydrolysis.

With the impetus of modular and efficient synthesis, we introduced olefin cross-metathesis (CM) in polysaccharide functionalization. Using Grubbs type catalyst, cellulose esters with terminally olefinic side chains were reacted with various CM partners including acrylic acid, acrylates and acrylamides to afford families of functionalized cellulose esters. Molar excesses of CM partners were used in order to suppress potential crosslinking caused by self-metathesis between terminally olefinic side chains. Amide CM partners can chelate with the ruthenium catalyst and cause low conversions in conventional solvents such as THF. While the inherent reactivity toward CM and tendency of acrylamides to chelate Ru is influenced by the acrylamide N-substituents, employing acetic acid as a solvent significantly improved the conversion of certain acrylamides. We observed that the CM products are prone to crosslinking during storage, and found that the crosslinking is likely caused by free radical abstraction of γ-hydrogen of the α,β-unsaturation and subsequent recombination. We further demonstrated successful hydrogenation of these α,β-unsaturated acids, esters, and amides, thereby eliminating the potential for radical-induced crosslinking during storage.

The α,β-unsaturation on CM products can cause crosslinking due to γ-H abstraction and recombination if not reduced immediately after reaction. Instead of eliminating the double bond by hydrogenation, we described a method to make use of these reactive conjugated olefins by post-CM thiol-Michael addition. Under amine catalysis, different CM products and thiols were combined and reacted. Using proper thiols and catalyst, complete conversion can be achieved under mild reaction conditions. The combination of the two modular reactions creates versatile access to multi-functionalized cellulose derivatives.

Compared with conventional reactions, these reactions enable click or click-like conjugation of functional groups onto cellulose backbone. The modular profile of the reactions enables clean and informative structure-property relationship studies for ASD. These approaches also provide opportunities for the synthesis of chemically and architecturally diverse cellulosic

polymers that are otherwise difficult to access, opening doors for many other applications such as antimicrobial, antifouling, in vivo drug delivery, and bioconjugation. We believe that the cellulose functionalization approaches we pioneered can be expanded to the modification of other polysaccharides and polymers, and that these reactions will become useful tools in the toolbox of polymer/polysaccharide chemists.

General Audience Abstract

Oral delivery of drug compounds by pills, capsules and other forms is one of the most convenient and practical ways for patients. One of the keys to determine whether a drug compound can be taken orally is its water solubility, as the digestive tract mainly uptakes dissolved molecules into the circulatory system. However, a lot of drug compounds do not suit this means as they have very low water-solubility due to their low tendency to mix with water. It makes them even less soluble if the pharmaceutically active molecules tend to crystallize.

Cellulose is a long chain polymer of linked glucose molecules, and is the most abundant natural polysaccharide on earth. It is the main component of cell walls of wood, cotton, and other plants, which provides plants with sufficient structural strength. Certain bacteria and animals (e.g. tunicate) can also produce cellulose. Cellulose is an attractive material from oral drug delivery as it is non-toxic, biodegradable and renewable. The Edgar group has developed a new technique to make oral drug delivery easier by using cellulose derivatives as an excipient formulated with the active ingredient of a medication. By suspending a drug in a matrix of a cellulose derivative, it prevents the drug from forming insoluble crystals on the one hand, and protects the drug molecules from the harsh environment in the stomach on the other. Overall, the formulation enhances the drug concentration in the digestive tract and increases its bioavailability.

However, it is difficult, if not impossible, to find one polymer matrix that works well for most drugs, as medications have broadly diverse chemical structures, physical properties, and delivery requirements. For cellulose derivatives, the structure and functionalities the polymers bear play an important role as they can interact with both drug molecules and the digestive tract environment to facilitate the successful delivery of the medications. To this end, it is important to investigate and understand what structure or structures (including functional groups on the polymer) are critical in drug delivery so that one can develop the next generation of polymer matrices for oral drug delivery. A big challenge in this study is the limited availability of synthetic methods, which have limited the accessibility of structurally and functionally diverse cellulose derivatives for clean and informative structure-property relationship study.

The works in this dissertation focus on the development of novel synthetic methods in chemical modification of cellulose and cellulose esters. Unlike conventional methods, which, more often than not, require long reaction time, harsh reaction condition, and usually afford

limited functionalized products, the new methods pioneered in this dissertation offer much simpler ways towards a wide variety of polymers using cellulose as a starting material, with short reaction time, mild conditions, and multiple choices of functional groups. These approaches provide a new platform for making and testing the next generation of polymer matrices for oral drug delivery. Moreover, with the approaches, we can make a lot of cellulose derivatives that are otherwise inaccessible. These new polymers are potentially useful in applications such as coating, cosmetics, food additives and so on.

Dedication

To my dear wife and our unborn baby.

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There are not so many *four years* in one's whole lifetime, and even less if you confine to the ones in which reside great meanings and unforgettable memories. I am lucky enough to spend such a four-year at Virginia Tech, where I have studied from respectful professors, collaborated with excellent peers, embraced challenges and achieved joys.

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Attribution

Several colleagues aided in data collection and research for the chapters within this dissertation. A brief description of their contributions is included here.

Dr. Kevin J. Edgar was the principal investigator and was a primary contributor in the planning, organizing, and directing of the projects described in this dissertation.

Dr. John Matson provided tremendous help in the establishment of the cross-metathesis works.

All the research works are based on previous findings and experiences of the Edgar group and the Taylor group.

Chapter 2: Literature review: "Click" reactions in polysaccharide modification.

Dr. John Matson helped to review and edit the manuscript, and gave useful advice and discussions on the review manuscript.

Chapter 3: Hydroboration-oxidation: A chemoselective route to cellulose ω-hydroxyalkanoate esters.

Ms. Emily A. York as a REU summer student at that time contributed part of the synthesis work and served as a coauthor on this paper.

Mr. Shu Liu at Virginia Tech (the Edgar group) assisted in data collection for part of the results in Chapter 3 and served as a coauthor on this paper.

Dr. Sue Mecham, Mr. Mark Flynn and Ms. Shreya Roy Choudhury of Virginia Tech helped with SEC analyses.

Chapter 4: Olefin cross-metathesis as a source of polysaccharide derivatives: Cellulose ω-carboxyalkanoates.

Dr. John Matson is a collaborator and served as a coauthor on this paper. His advice on CM catalyst and CM partner choice greatly prompted the progress of this work and the work in Chapter 5.

Mr. Evan Margaretta (the Long group) and Ms. Alice Savage (the Turner group) of Virginia Tech assisted to perform SEC analyses.

Chapter 5: Olefin cross-metathesis, a mild, modular approach to functionalized cellulose esters.

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Chapter 6: Synthesis of amide-functionalized cellulose esters by olefin cross-metathesis.

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Chapter 7: Multifunctional cellulose esters by olefin cross-metathesis and thiol-Michael addition.

Ms. Shreya Roy Choudhury of Virginia Tech assisted with SEC analyses and and she is a coauthor on the manuscript submitted

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Rules for Polymer Abbreviations:

The rules for polymer abbreviation/nomenclature in this dissertation are as follows. For terminally olefinic cellulose esters, e.g. CA-Un067 and CA-Pen079, *CA* denotes cellulose acetate; *Un* denotes undec-10-enoate and *Pen* denotes pent-4-enoate, while the numbers *067* and *079* indicate DS of each olefinic side chain. Other polymers starting from cellulose acetate propionate (CAP) or cellulose acetate butyrate cellulose (CAB) follow the same rule. For hydroboration-oxidation products (**Chapter 3**), the *Hb* is used to indicate the reaction, e.g. Hb-CA-Un067. For cross-metathesis products (**Chapter 4, 5, 6,** and **7**), e.g. CA-Un067-XX, *CA-Un067* indicates the terminally olefinic cellulose ester, while *XX* stands for the CM partner used in the reaction e.g. *AA* denotes acrylic acid and *BA* is benzyl acrylate. For thiol-Michael addition products (**Chapter 7**), e.g. CA-Un067-BA-YY, *CA-Un067-BA* indicates that the thiol-Michael substrate used is from CM product CA-Un067-BA, while *YY* stands for the thiol donor employed e.g. 3MPA denotes 3-mercaptopropionic acid.

Chapter 1: Research Introduction

Oral drug delivery has many advantages such as good patient compliance, non-invasive administration, and convenience over other administration routes. However, for many hydrophobic drugs and drug candidates (e.g. BCS II drugs), oral delivery of these drugs is more often than not challenging as one tends to achieve low solubility and thus low bioavailability of the drugs due to the high tendency to (re)crystallize during storage and administration. Amorphous solid dispersion (ASD) formulation technique has proven promising to address this issue. In a typical ASD formulation, an originally crystalline drug is dispersed molecularly in a polymer matrix, which keeps the drug molecules from crystallization during storage and administration. Cellulose, the most abundant natural polymer on earth, has played an important role in industries including pharmaceuticals, cosmetics, and coatings. Previous studies showed that certain cellulose derivatives were potent inhibitors of drug crystallization and might be potential ASD polymers for oral drug delivery.[1-3] To have a better understanding of the structure-property relationship, and to design cellulose derivatives with better performance for these applications, it is critical to be able to synthesize cellulose derivatives with various functionalities and structural architectures.

An overarching theme of the current research is centered on exploring novel approaches for cellulose functionalization, in the hope that these strategies would not only provide a broad spectrum of cellulose derivatives that are otherwise not accessible, but also would enable sound structure-property relationship studies for various applications. Chemical modification is one of the major means to provide cellulosic products with better-tuned physical and/or chemical properties. To date, cellulose and polysaccharide functionalization has heavily relied on conventional methods such as esterification and etherification which, although they have

advantages including straightforward reaction routes and easy access to reaction reagents, suffer from issues such as harsh reaction conditions, limited choice of reagents, and limited types of functionality that can be grafted onto the cellulose backbone. To that end, this research will explore several chemistries that are mild, chemoselective, and efficient, in order to introduce these chemistries to polysaccharide community and at the same time prepare chemically and structurally more diversified cellulose derivatives for oral drug delivery and corresponding in-depth structure-property relationship studies.

Olefins will be heavily utilized in this research as reactive handles for further chemical modification reactions. On the one hand, olefins are comparatively stable and compatible with other functionalities (e.g. acid chlorides and carboxylic acids) and reactions (e.g. esterification and esterification, which are commonly employed in cellulose modification). As a result, it is convenient to graft olefins onto the cellulose backbone without significant side reactions, and without requiring special care during storage. On the other hand, olefins are reactive in certain reactions such as hydroboration-oxidation, hydroboration-amination, olefin metathesis, thiol-ene reaction, and thiol-Michael reaction. In a designated reaction under proper conditions, cellulose with olefinic side chains will react chemoselectively at the olefin sites and afford corresponding functional groups. This property makes it possible to produce cellulose derivatives with (almost) identical molecular weight, substitution pattern, and degree of substitution, differing in terminal functional groups. Thus, this promises to be an excellent platform not only for various families of new polymers, but also for structure-property relationship studies that isolate the effects of changing the terminal functional group. In this research, we will focus on several reactions, including hydroboration-oxidation to transform terminal alkenes to hydroxyl groups, olefin cross-metathesis (CM), which is more powerful towards a variety of functionalities, and thiol-

Michael addition, which makes use of the α,β-unsaturation after CM to append secondary functionalities onto the side chains of cellulose derivatives.

An outline for this dissertation is as follows: **Chapter 2** will introduce some fundamentals in polysaccharides and polysaccharide modification and give a review of novel approaches in polysaccharide modification. The efforts of employing click reactions (including copper catalyzed alkyne/azide cycloaddition (CuAAC), metal-free [3+2] cycloaddition, Diels-Alder reaction, oxime click, thiol-Michael reaction, and thiol-ene reaction) in polysaccharide modification will be elaborated in this chapter. Olefin cross-metathesis (CM) will also be introduced in this chapter. Although it does not satisfy every published requirement to be considered a click reaction, we describe it as click-like, and it can afford post-modification products in an efficient and modular manner under mild conditions. **Chapter 3** will then present our efforts towards synthesis of cellulose ω-hydroxyalkanoate esters by hydroboration-oxidation. This chapter will discuss the potential problems of hydroboration-oxidation in the modification of cellulose esters, which involves hydrolysis and reduction of esters under typical conditions, and will describe our efforts to circumvent these problems. From **Chapter 4** to **Chapter 6**, olefin cross-metathesis as a new tool in polysaccharide modification will be discussed using cellulose esters with terminally olefinic side chains as substrates. As a proof-of-concept, **Chapter 4** will focus on the reactions between terminally olefinic cellulose esters and acrylic acid towards ω-carboxyalkenoates. Reaction conditions including type of catalyst, solvent, temperature and time will be discussed in this chapter. We will describe and rationalize the crosslinking potential of the cross-metathesis products. Methods to avoid crosslinking will also be proposed in this chapter. **Chapter 5** will then expand the cross-metathesis partners from acrylic acid to acrylates. In this chapter, we try to convince the readers that olefin cross-metathesis is a mild and modular

approach towards cellulose functionalization. We also eliminate the α, β-unsaturation after cross-metathesis by hydrogenation, which eliminates the cause of the crosslinking. **Chapter 6** will describe difficulties encountered when we tried to expand the CM partners to acrylamides. Chelation of CM partners with the Ru catalyst, interactions between solvents, CM partners, and the catalyst, as well as the influence of substituents on CM partners will be discussed in this chapter. We propose in this chapter a more efficient solvent (acetic acid) for CM with certain acrylamides, as well as a more universal hydrogenation method (*p*-toluenesulfonyl hydrazide) to eliminate the α, β-unsaturation. **Chapter 7** will introduce a double modification strategy, i.e. cross-metathesis followed by thiol-Michael addition for further functionalization of cellulose esters. The combination of the two modular reactions makes it possible to produce cellulose derivatives with more functional diversities. At last, **Chapter 8** will conclude with a summary of the research results discussed in this dissertation and give some suggestions for future work.

(1) Ilevbare, G. A.; Liu, H. Y.; Edgar, K. J.; Taylor, L. S. *Crystal Growth & Design* **2012**, *12*, 3133.
(2) Ilevbare, G. A.; Liu, H. Y.; Edgar, K. J.; Taylor, L. S. *Crystal Growth & Design* **2013**, *13*, 740.
(3) Ilevbare, G. A.; Liu, H. Y.; Edgar, K. J.; Taylor, L. S. *Mol. Pharmaceutics* **2013**, *10*, 2381.

Chapter 2: Literature review: "Click" reactions in polysaccharide modification

(Adapted with permission of Elsevier Ltd.: Meng X. and Edgar K. J., "Click" reactions in polysaccharide modification. Prog. Polym. Sci. 2016, 53, 52-85.)

Abstract

Polysaccharide chemistry is enjoying accelerating development thanks to advances in synthetic techniques, biochemistry and solvents, which enable polysaccharide materials to be useful in a variety of demanding applications. Among the synthetic advances, click chemistry has reconfigured the realm of polysaccharide modification that previously was dominated by conventional synthetic approaches such as esterification and etherification. "Click" reactions provide mild, modular, and efficient modification pathways, and equally importantly allow us to synthesize derivatives with novel functionality, architecture, and properties, that are otherwise difficult to obtain via conventional methods. Herein, we review application in polysaccharide modification of six groups of click reactions; CuAAC (copper catalyzed alkyne/azide cycloaddition), metal-free [3+2] cycloaddition, Diels-Alder reaction, oxime click, thiol-Michael reaction, and thiol-ene reaction, as well as one click-like reaction that is the subject of our own research, olefin cross-metathesis.

Nomenclature

AAC: azide-alkyne cycloaddition; BCN: bicycle[6.1.0]nonynes; BCN(ene): Bicyclo[6.1.0]nonene BARAC: biarylazacyclooctynones; CD: cyclodextrin; CDI: 1,1'-carbonyldiimidazole; CM: cross-metathesis; CMC: carboxymethyl cellulose; CNC: cellulose nanocrystal; CNP: chitosan nanoparticle; CS: chitosan; CTA: cellulose triacetate; CuAAC: copper catalyzed azide-alkyne cycloaddition; DA: Diels-Alder reaction; DCC: N,N'-dicyclohexylcarbodiimide; DIBAC: dibenzoazacyclooctynes; DIBO: dibenzoazacyclooctynes; DIFO: difluorocyclooctynes; DIPEA: N,N-Diisopropylethylamine; DMAP: 4-dimethylaminopyridine; DMF: dimethylformamide; DMPA: 2,2-Dimethoxy-2-phenylacetophenone; DMSO: dimethyl sulfoxide; DOTA: 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; DTP: 3,3'-dithiodipropionic acid; DTT: 1,4-dithio-DL-threitol; DVS: divinyl sulfone; EDAC: 1-ethyl-3-(3-dimethylaminopropyl-carbodiimide); EDC: ethyl(dimethylaminopropyl) carbodiimidazole; FITC: Fluorescein isothiocyanate; HA:

hyaluronic acid; hDA: hetero Diels-Alder reaction; HEMA: hydroxyethyl methacrylate; HOBt: 1-hydroxybenzotriazole; iEDDA: inverse electron demand Diels-Alder reaction; ISA: imidazole-1-sulfonyl azide hydrochloride; MES: 2-(N-morpholino)-ethanesulfonic acid; NBS: N-bromosuccinimide; NHS: N-hydroxysuccinimide; NMP: N-methyl-1-pyrrolidone; PBLG: poly(γ-benzyl L-glutamate); PBS: phosphate-buffered saline; PDMAEMA: poly(2-dimethylaminoethyl methacrylate); PEG: poly(ethylene glycol); PMA: 2-propyn-1-yl methacrylate; PMDETA: N,N,N',N',N"-pentamethyldiethylenetriamine; PMT: polymer modified tetrazine; PNIPAM: poly(N-isoproylacrylamide); PPMA: poly(2-propyn-1-yl methacrylate); rDA: retro Diels-Alder reaction; ROS: reactive oxygen species; SM: self-metathesis; SPAAC: strain-promoted azide-alkyne cycloaddition; TBA: tetrabutylammonium; TCO: trans-cyclooctene; TEA: triethylamine; TFA: trifluoroacetic acid; TFMSA: trifluoromethanesulfonyl azide; THF: tetrahydrofuran; TsCl: 4-toluenesulfonyl chloride; Tz: tetrazine; VS: vinyl sulfone

2.1 Introduction

Polysaccharides (including cellulose, chitosan, alginate, dextran, hyaluronic acid and others) are among the most abundant natural polymers on earth. They and their modified derivatives are under extensive investigation and are currently used for applications such as coatings[1], drug delivery[2,3], and biomedical materials[4-6], due to the sustainability of biopolymers, the biological functions they possess, and also to the fact that the structure and properties of these biopolymers are readily tunable. Chemical modification is one important approach to tailor polysaccharide structure and properties. By chemical modification of homogeneously dispersed polysaccharide molecules or on the surfaces of polysaccharide materials, derivatives bearing different functional groups and conjugates can be obtained. Modification of polysaccharides also generates a variety of derivatives that are capable of forming special architectures such as nanogels[7], hydrogels[8], and micelles[9]. From this point of view, chemical modification imparts desired properties to the polysaccharide materials so that they meet the requirements of specific applications.

Conventional modification approaches generally involve esterification or etherification, taking the advantage of the straightforwardness of the reactions and the comparatively easy access to many esterification and etherification reagents. Other modification methods including

nucleophilic displacement reactions, oxidation, and (controlled) free radical polymerization have also been commonly employed. Certainly these synthetic pathways have enlarged the family of polysaccharide derivatives. For example, esterification of polysaccharides has contributed remarkably to cellulose and polysaccharide chemistry in the last few decades due to the development of new acylation methods and unconventional solvents.[10] Investigation of regioselective reactions and protection/deprotection groups, on the other hand, provides options for regioselectively modified polysaccharide derivatives with well-controlled structures, and permits deeper understanding of structure-property relationships of polysaccharide derivatives[11]. While such methods are very useful and are still contributing to the existence of entire industries, they are limited in scope. Typically esterification involves harsh reaction conditions (e.g. strongly acidic catalysts) that are incompatible with sensitive functional groups on either polysaccharides or the acylation reagents. In the absence of protecting groups, simple esterification is also incompatible with difunctional reagents such as dicarboxylic acids or reagents with both carboxylic acid and hydroxyl groups, which could lead to undesired crosslinking or uncontrolled polymerization[12]. The advent of acyl activation reagents, e.g. N,N'-dicyclohexylcarbodiimide (DCC), has allowed the performance of esterification under milder conditions, but this mild esterification is still not useful with difunctional reagents. Etherification typically involves strongly basic conditions (e.g. NaOH or NaH), and so is incompatible with base-sensitive moieties and mostly incompatible with difunctional reagents. Further, long reaction times, tedious steps, and modest yields are also at times associated with these conventional methods.

The concept of "click chemistry"[13], first coined by Sharpless and his coworkers, has had a huge impact on the chemistry community[14-18], detailed elaboration of which is beyond the scope of this review. To qualify as "click" chemistry, a reaction must fulfill most, if not all, of the requirements listed below. The reaction must "be modular, wide in scope, give very high yields, generate only inoffensive and easily removable byproducts, and be stereospecific"; and the process should also fulfill "simple reaction conditions and product isolation, readily available reagents, and the use of no solvent or benign ones". The concept of modular, facile and economical synthesis of structurally and functionally diverse molecules has also drawn attention from polysaccharide chemists, and has brought substantial progress to this field. Not only can "click" reactions avoid the aforementioned limitations of conventional synthetic pathways, but

they also can rapidly synthesize molecules with diverse functional appendages. Herein, we review some of the most commonly used "click" reactions in polysaccharide chemistry, including the well-known azide-alkyne Huisgen cycloaddition, Diels-Alder reaction, thiol-ene and thiol-Michael reactions, and the oxime click reaction. As several new reactions fulfilling the criteria of click chemistry have been uncovered in the last decade, we also reviewed the introduction of these new click reactions to the field of polysaccharide chemistry, including two metal-free [3+2] cycloaddition reactions, and the inverse electron-demand Diels-Alder reaction. We focus in this review mainly on the chemistry of these reactions, and try to provide to readers a guide for performing such reactions. At the same time, the extraordinarily diverse and controllable structures and functionalities of the click reaction products are demonstrated. We also compare the strengths and limitations of each click reaction, hoping to provide some guidance to future polysaccharide researchers selecting tools from this toolkit. Although olefin cross-metathesis has not previously been considered a "click" reaction in either organic or polymer chemistry, we find it has many elements of click reactions under certain circumstances (i.e. electron-rich type I terminal olefins reacting with electron deficient type II or III olefins)[19,20]. We present here the concept that olefin cross-metathesis of polysaccharide derivatives is a novel "click-like" reaction, hoping that this facile, efficient and modular reaction could be added in the toolbox of polysaccharide chemists for chemical modification.

2.2 Copper catalyzed azide-alkyne cycloaddition (CuAAC)

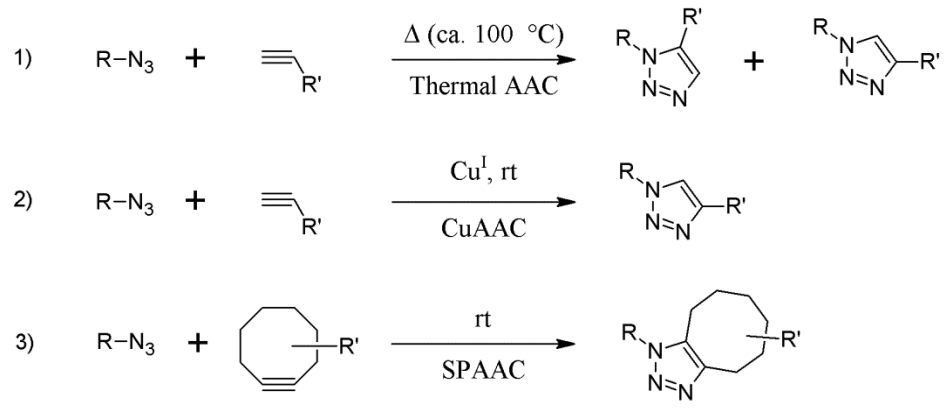


Figure 2.1. Three versions of azide-alkyne cycloaddition: 1) the classic thermal azide-alkyne cycloaddition; 2) Cu-catalyzed azide-alkyne cycloaddition; and 3) the metal-free strain-promoted

azide-alkyne cycloaddition. Reproduced with permission from Ref. 21[21]. Copyright 2011, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Since the introduction of the "click" reaction concept by Kolb, Finn, and Sharpless in 2001, Huisgen 1,3-dipolar azide-alkyne cycloaddition (AAC)[22] has been featured as the flagship of all click reactions. Three versions of AAC (**Figure 2.1**) were developed, i.e. the classic thermal AAC, the broadly used Cu-catalyzed azide-alkyne cycloaddition (CuAAC), and the metal-free strain-promoted azide-alkyne cycloaddition (SPAAC), which will be discussed separately in the next section. Although the thermal version of AAC shares the features of the others including high reliability, bioorthogonality (the ability of reactants to react rapidly and selectively only with each other, usually inside living systems, without interfering with other functionality[23]) and broad tolerance to diverse functional groups, the facts that it has a low reaction rate, requires comparatively high temperatures, and leads to two different isomerized products (1,4-disubstituted 1,2,3-triazoles and 1,5-disubstituted 1,2,3-triazoles) limit its broad application. It was not until the discovery of the Cu-catalyzed version of this reaction by the Meldal[24] and Sharpless[25] groups that the reaction started to gain considerable attention. As the role of copper in the reaction has been under intensive investigation, and is still in dispute, the discussion of plausible mechanisms[26-28] will not be addressed in this review. However, Cu-(I) complexes are able to catalyze the reaction, providing remarkably high rates under highly diverse conditions of solvent, temperature, nature of catalyst precursor, and substrate structure.

It didn't take long for polysaccharide chemists to realize the practicality and reliability of this reaction in polysaccharide modification. In 2006, the Hafren and Cordova group[29], the Heinze group[30] and the Shinkai group[31] independently reported the application of CuAAC in polysaccharide derivatization. In this section we summarize the representative strategies, especially in the pre-click polysaccharide functionalization, applications of this reaction in homogeneous and heterogeneous modification, as well as crosslinking of polysaccharides, and also the limitations of CuAAC.

2.2.1 Pre-click functionalization

2.2.1.1 Azide on backbone

A preliminary step towards the CuAAC reaction is to functionalize the polysaccharide with either azide or alkyne. One widely used approach is to synthesize 6-azido-6-deoxy-polysaccharides (**Figure 2.2**). For many polysaccharides such as cellulose, curdlan and chitosan (CS) that have free primary hydroxyl groups at C-6, this strategy can be accomplished by low-temperature tosylation of the polysaccharide at C-6, followed by nucleophilic tosylate displacement with azide (NaN_3)[30,32]. Alternatively, azide moieties can be introduced to the C-6 position of polysaccharides[31,33] by initial activation of the primary hydroxyl group using triphenylphosphine (Ph_3P), subsequent bromination with tetrabromomethane or N-bromosuccinimide (NBS, which also serve to activate Ph_3P towards nucleophilic attack by OH group to form an alkoxyphosphonium salt intermediate[34]), followed by azide substitution on the 6-bromo-6-deoxy derivatives. One particular benefit to this approach is that the reactions occur with great selectivity at the primary hydroxyl group, which guarantees the regioselectivity of the derivatives[11]. These reactions, combined with the CuAAC reactions, lead to regioselective, quantitative, and modular polysaccharide modification.

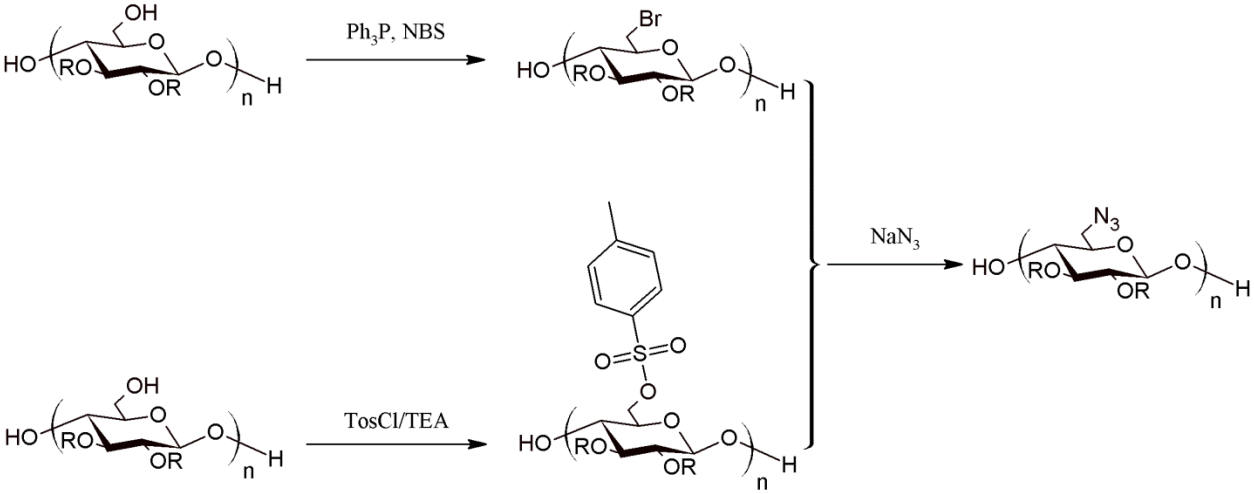


Figure 2.2. Two pathways for synthesis of 6-azido-6-deoxy polysaccharide derivatives (using cellulose as example).

Another approach to azido-polysaccharide was synthesis of 2-azido-2-deoxycellulose from chitosan. This derivative has the potential to become a platform in regioselective

modification, especially as an alternative to its 6-azido-6-deoxy counterpart for structure-property relationship studies. One method developed by Zhang and coworkers[35] afforded 2-azido-2-deoxychitosan with high degree of conversion to azide (95%) using trifluoromethanesulfonyl azide (TFMSA). However, multiple steps, harsh reaction conditions, and long reaction times may impact the structural fidelity of the polymer (e.g. molecular weight, which the authors did not report) and limit the practicality of this method. Kulbokaite et al.[36] compared three procedures (**Figure 2.3**) for azidation of chitosan using sodium nitrite and sodium azide, TFMSA, and imidazol-1-sulfonyl azide hydrochloride (ISA) as azidation agents, and found that TFMSA and ISA were suitable for N-azidation of chitosan. However, the maximal degrees of azidation of chitosan obtained from these two methods were only 40% and 65% respectively.

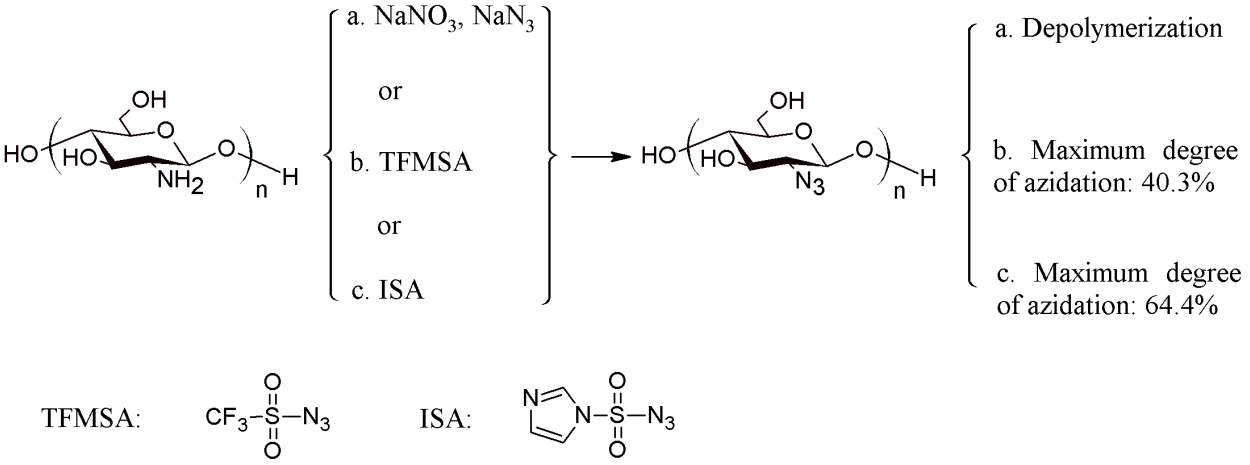


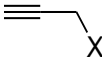
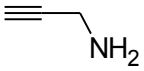
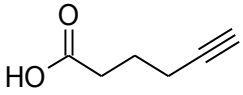
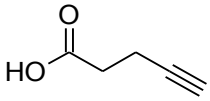
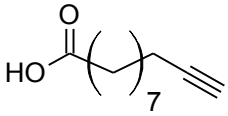
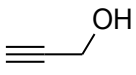
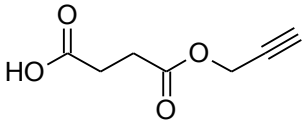
Figure 2.3. N-azidation of chitosan by three procedures. Adapted with permission from Ref. 36[36].

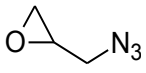
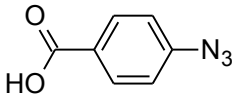
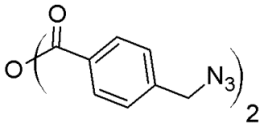
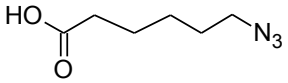
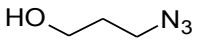
2.2.1.2 Tethered azides or alkynes

More diverse azide or alkyne functionalized polysaccharides were obtained by linking small azide- or alkyne-bearing groups to polysaccharide backbones. The most typical methods are through nucleophilic substitution reactions (e.g. etherification) or esterification of backbone hydroxyl groups (amine groups on amino-polysaccharides to form secondary amine and amide are also employed) with azide or alkyne moieties (**Table 2.1**). While most of the nucleophilic substitution reactions require the assistance of strong bases[37-41] like sodium hydride, or the pre-substitution/activation of the hydroxyl group of the polysaccharide by a good leaving group[42],

the success of esterification reactions largely depends on catalysts or activation agents including 1,1'-carbonyldiimidazole (CDI), DCC, and ethyl(dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS). Azide functionality can also be introduced stepwise by first introducing a halogen functionalized side chain to the polysaccharide backbone followed by nucleophilic substitution of the halogen with azide[43-45]. These classical reactions are synthetically straightforward, and can usually give moderate to high degree of substitution (DS) values, largely depending on the stoichiometry. However, unless combined with protecting groups to control regiochemistry, these reactions are often not inherently chemo- or regioselective.

Table 2.1. Alkynes and azides commonly used to functionalize polysaccharides.

Reagent	**Structure**	**General reaction condition**	**Polysaccharides**
Propargyl halide		Base (NaH, NaOH), 40-50 ºC, 72-96 h	Cellulose[37-39], chitosan[40], starch[41]
Propargyl amine		80 ºC, 24 h in DMSO	Cellulose (6-tosyl cellulose)[42]
5-Hexynoic acid		(S)-tartaric acid, 110 ºC, 6 h	Cellulose[29]
Pent-4-ynoic acid		EDC/NHS, r.t., 16 h	Chitosan[46]
Undec-10-ynoic acid		TsCl/pyridine, 80 ºC, 36 h	Cellulose[47]
Propargyl alcohol		CDI	Dextran[48]
Propargyl 3-succinate		EDC/HOBt[a], r.t., 24 h	Chitosan[49]

1-Azido-2,3-epoxypropane		Base (NaOH), 30 ºC, ~20 h	Cellulose[50], dextran[51]
4-Azidobenzoic acid		DCC, 30-50 ºC, 24-72 h	Chitosan[38]
4-Azidomethylene benzoic anhydride		TEA[b]/DMAP[c], r.t., overnight	Cellulose surface[52]
6-Azidohexanoic acid		CDI	Dextran[53]
3-Azido-1-propanol		CDI	Dextran[48]

[a]HOBt: 1-hydroxybenzotriazole; [b]TEA: triethylamine; [c]DMAP: 4-dimethylaminopyridine

Making use of previously regioselectively attached functional groups (e.g. carboxylic acid group on carboxymethyl cellulose (CMC)) permits chemo- or regioselective polysaccharide functionalization without employing protection/deprotection techniques. One widely explored strategy is the activation of carboxylic acid groups in certain polysaccharides, including hyaluronic acid (HA) and CMC using coupling agents such as EDC/NHS, followed by the addition of azido- or alkynyl amines[54,55] (**Figure 2.4**). There was a report of chemoselective synthesis of an azido chitosan derivative[56], which was prepared through chemoselective N-bromophthaloylation of chitosan and subsequent azidation.

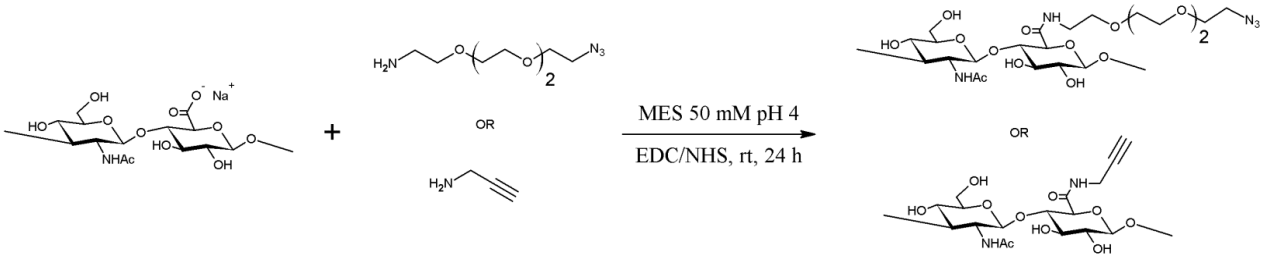


Figure 2.4. Schematic presentation of the reaction between HA and amine-functionalized azide or alkyne with the assistance of coupling agent EDC/NHS.

The reducing and non-reducing ends of polysaccharides can be useful sites for chemoselective functionalization with azide and alkyne moieties. Kamitakahara's group developed methods of converting the reducing end of cellulose triacetate (CTA) to azides[57-59] (**Figure 2.5a**) and non-reducing end of 2,3,6-tri-O-methyl-cellulose to alkynes[58] (**Figure 2.5b**). The corresponding products bearing CuAAC handles are good building blocks for cellulose-based diblock copolymers (**Figure 2.5c**). However, the reaction mechanism restricted viable substrates to peracylated and permethylated polysaccharides, and the degrees of polymerization (DP) of the final products were low. A simpler and more applicable method was reported independently by Bernard et al.[60] and Lecommandoux et al.[61,62] As shown in **Figure 2.6a**, an alkyne group was introduced to the reducing end of dextran by reacting with propargylamine to form a Schiff base, which was reduced by sodium cyanoborohydride in a one-pot reaction to give a more stable amine product. As a majority of polysaccharides and polysaccharide derivatives have aldehyde available in equilibrium at the reducing ends, reductive amination is a broadly applicable approach for introducing azide or alkyne handles to the reducing end of polysaccharides. Besides the abovementioned methods, Yamaguchi et al.[63] reported an alternative pathway with the help of *endo*-β-xylosidase, which cleaved the β-xyloside linkage of the substrate (peptide chondroitin 6-sulfate) between the reducing end xylose and the hydroxyl group of the side chain of the peptide, and catalyzed transglycosylation at the same time in the presence of propargyl alcohol. Although limited to only a few polysaccharides, this green synthesis approach is intriguing. These end-functionalization methods contributed to new copolymeric architectures such as diblock copolymers[58,61,63], and comb-shaped graft-copolymers[59], which will be described in detail in the following contexts.

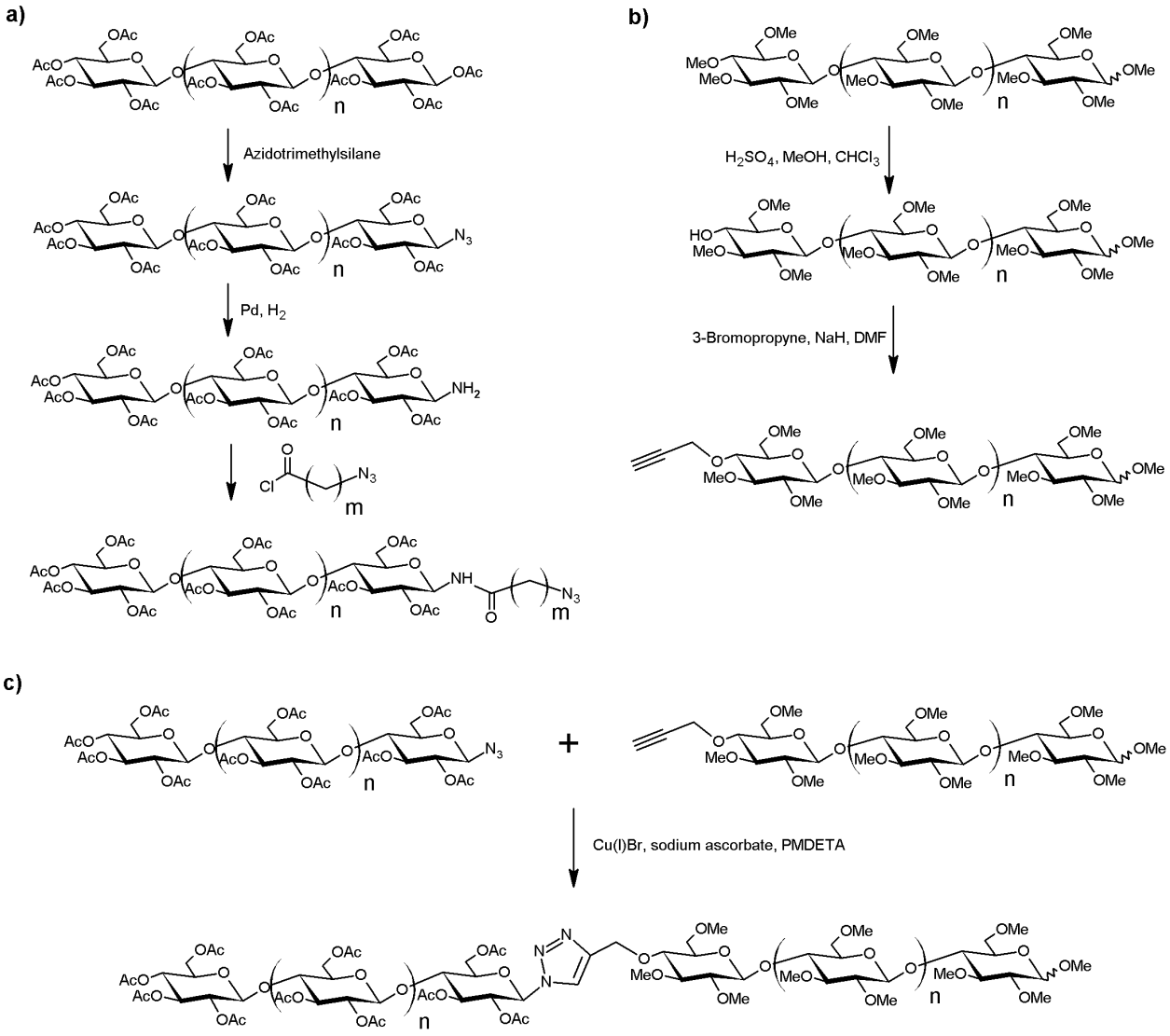


Figure 2.5. Synthetic pathways for a) peracetylated cellulosic glycosyl azides; b) methyl 2,3,6-tri-O-methyl celluloside having a propargyl group at C-4 of non-reducing end; c) an example of CuAAC between a cellulosic glycosyl azide and a propargyl cellulose to afford a block copolymer. Adapted with permission from Ref. 58[58]. Copyright 2012, Springer Science+Business Media B.V.

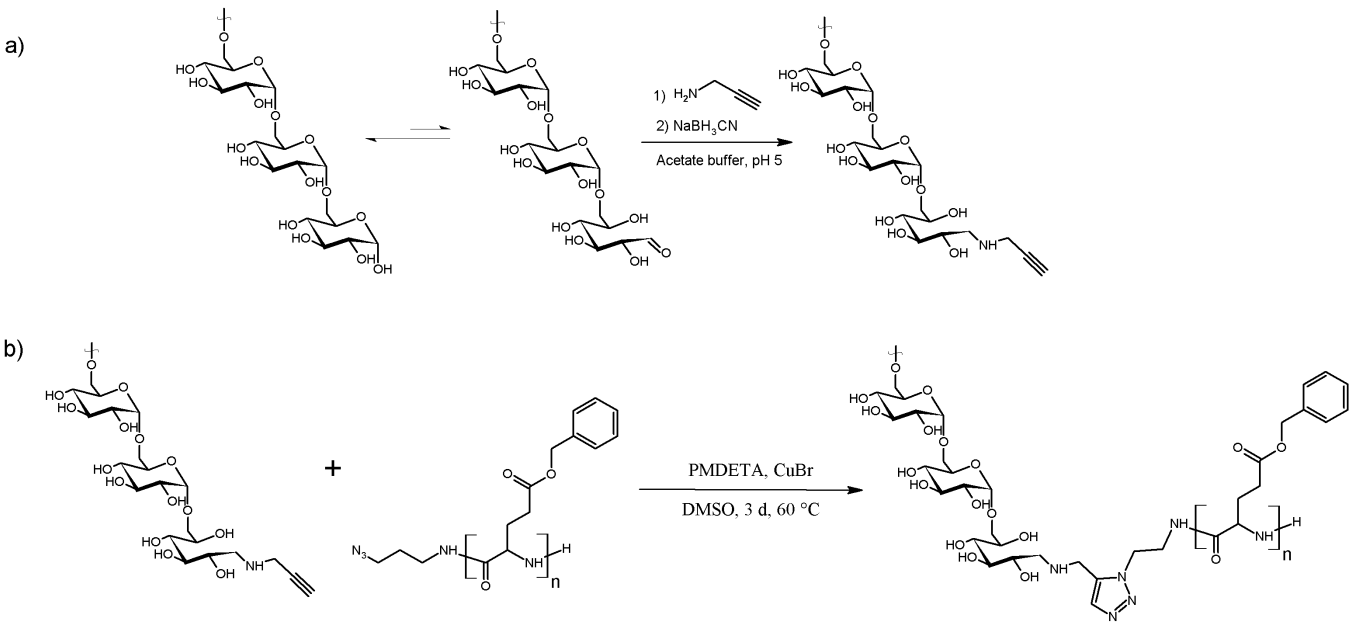


Figure 2.6. Synthesis of a dextran-block-poly(γ-benzyl L-glutamate) copolymer via CuAAC. a) Synthesis of end-functionalized dextran with an alkyne group by the reductive amination of dextran with propargylamine. b) Block coupling with azido end-functionalized PBLG by click chemistry. Adapted with permission from Ref. 61[61]. Copyright 2009, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

2.2.2 1,3-Dipolar azide-alkyne cycloaddition

The key to the cycloaddition is the copper (I) catalyst, which contributes to moderate reaction temperature and more importantly controlled 1,4-regioselectivity (only 1,4-disubstituted 1,2,3-triazole products) of the reaction[24,25]. Several protocols to introduce Cu^I into the system were developed[24,25,64] and widely employed in click reactions of polysaccharide derivatives, including a) formation of Cu^I by addition of Cu^I salt (e.g. CuBr)[61], and b) generation of Cu^I by reduction of Cu^{II} salt (e.g. $CuBr_2$/ascorbic acid)[31]. Ligands, e.g. N,N,N′,N′,N″-pentamethyldiethylenetriamine (PMDETA)[36,49,60,61], were sometimes used to accelerate the reaction, likely by protecting the Cu^I ion from oxidation and disproportionation[65], although alkyne–azide cycloaddition can be effectively catalyzed under "ligand-free" conditions, in which solvents or bases[31] act as ligands.

As a robust reaction, CuAAC tolerates a variety of solvent systems and functional groups. Solvents including DMSO[31], DMF[45], NMP[31], THF[52], H_2O[29], aqueous HCl (caution that acid can acidify azide to form HN_3, which is volatile and highly toxic)[46], and ionic liquids[66] have

been reported in homogeneous and heterogeneous modification of polysaccharides. This tolerance has provided great versatility for the reaction in polysaccharide chemistry as the starting polysaccharide derivatives are usually difficult to dissolve in other common organic solvents like alcohols.

Tolerance of the reaction towards a wide scope of functional groups, on the other hand, has contributed to thriving families of polysaccharide derivatives through either homogeneous or surface modification strategies. The "click" of small molecular appendages[30,31] onto polysaccharide backbones has proven a great success, during which full conversion to triazoles can be achieved. For example, Liebert and Heinze found that high conversion (from 81 to 98%, depending on the alkynyl partner used) could be achieved in CuAAC of 6-azido-6-deoxy cellulose by applying a molar ratio (alkyne to azide) of 3:1 at a reaction temperature of 25 ºC in DMSO for 24 h[30]. Hasegawa et al. employed an excess of small molecule alkynyl compounds bearing different functional groups such as β-lactoside and pyrene to react with 6-azido-6-deoxy curdlan (26.7 ~ 1.8 : 1 in molar ratio) and achieved complete conversion to click products[31].

Encouraged by these results, derivatives with more diverse architectures including graft[36,44,46,59,67], block[61-63], and dendronized[37,68] copolymers have been synthesized via CuAAC. Copolymerization of two or more macromolecules may not only combine the properties of the polymers, but may also generate new properties. The CuAAC reaction provides a "graft onto" strategy, which gives more controllable and well-defined copolymers than the "graft from" counterpart. Commonly, side chain alkynylated or azidated polysaccharides were reacted with azido- or alkynyl-terminated synthetic polymers or dendrons under typical CuAAC conditions. Synthetic polymers such as PEG[36], PDMAEMA[44] and PNIPAM[44,46] have been grafted onto polysaccharide backbones via CuAAC click chemistry. As with small molecules, high coupling efficiency (above 80%) can be achieved under standard reaction conditions (e.g. DMSO, 40 ºC, 24 h), especially when a slight excess of the synthetic polymer moiety is used to ensure complete cycloaddition.

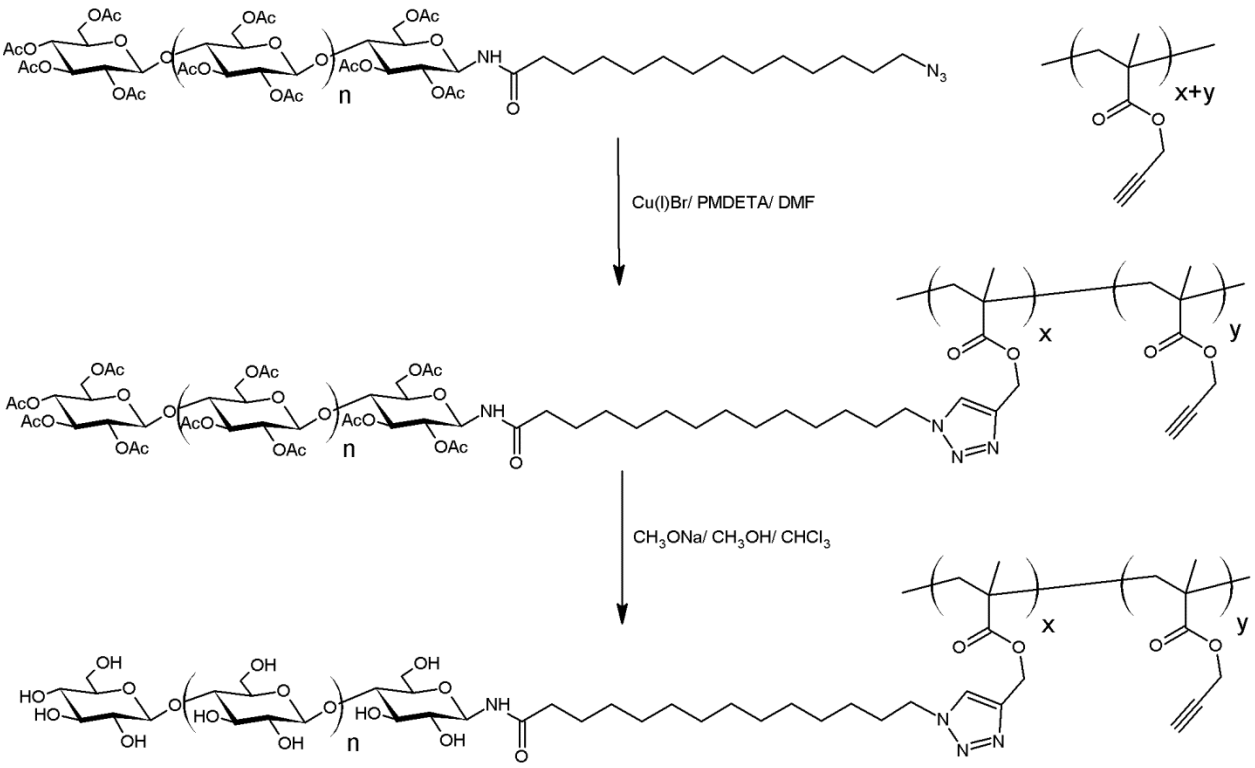


Figure 2.7. Preparation of poly(2-propyn-1-yl methacrylate)-g-cellulose triacetate (PPMA-g-CTA) and PPMA-g-cellulose by CuAAC. Adapted from Ref. 59[59] published by Elsevier.

The end-functionalization methods[58-63] discussed in the previous section, which generate azido- or alkynyl terminated polysaccharides, enable the synthesis of graft-co-polymers with polysaccharides as the side chains, or linear block copolymers containing polysaccharide segments. The Kamitakahara group prepared poly(2-propyn-1-yl methacrylate)-g-cellulose triacetate (PPMA-g-CTA) and PPMA-g-cellulose by CuAAC (**Figure 2.7**)[59]. With the ratio of PMA monomer (alkyne) to azido-CTA equaling to 1:1, quantitative formation of triazoles was observed and supported by the disappearance of the N_3 absorbance at 2120 cm^{-1} in FTIR spectra after the click reaction. Lecommandoux et al. introduced alkynyl groups to the reducing ends of dextran[61] and hyaluronic acid[62], and subsequently clicked the polysaccharide segments with an azido-terminated polypeptide to afford dextran-b-poly(γ-benzyl L-glutamate) (dextran-b-PBLG) and HA-b-PBLG respectively (**Figure 2.6b**). Polymersomes formed by self-assembly of the block copolymers showed controlled size and excellent stability, attributed to the hydrophilic polysaccharide segments, showing potential for drug delivery and other applications. Although it may seem more challenging to synthesize these copolymers by CuAAC due to higher steric

hindrance, coupling efficiency was satisfactory in most cases when an excess of one partner (e.g. mol ratio = 3:1) and longer reaction time (e.g. 48 h) were used.

Networks are another type of polymer architecture that can be obtained through the coupling of alkynes and azides. Crosslinking between the same[39,42,48,54,69] and different[38,41] polysaccharide species, and between synthetic polymers and polysaccharides[70] have been realized using similar conditions as used in CuAAC of polysaccharides with either small moieties or other polymers.

Heterogeneous modification by CuAAC of polysaccharide surfaces[47,52,71-76], such as those of cellulose nanocrystals (CNC), has also been intensively explored. As the principles are largely the same as for homogeneous modification mentioned above, this topic will not be discussed separately in this review.

2.2.3 Limitations

Undoubtedly, CuAAC has emerged as a useful tool in polysaccharide modification because of its modularity, efficiency and versatility. However, several limitations of this chemistry need to be taken into account. First of all, it is important to consider the toxicity of Cu(I), which is capable of mediating the generation of reactive oxygen species (ROS) from O_2 and thereby causing damage to living cells[77,78]. Although it might not be such a serious problem for certain polysaccharides under homogeneous conditions, where it may be possible to eliminate copper ions during purification, the removal of the biologically toxic Cu(I) compounds becomes problematic in the preparation of polymer networks. Furthermore, the tendency of aminopolysaccharides, such as chitosan[79], to chelate heavy metals makes the removal of Cu ions more difficult, under either homogeneous or heterogeneous conditions. Another problem that may arise with Cu(I) is depolymerization of polysaccharide. There have been a number of studies showing that ROS are able to induce oxidative cleavage of polysaccharide chains[80,81]. Cu ions were proven to increase the scission rate as Cu(I) reacts with H_2O_2 to generate $\cdot OH$[82]. Lallana et al. observed severe chain cleavage of the chitosan backbone after CuAAC, and rationalized the depolymerization as the result of hydroxyl radical ($\cdot OH$, a ROS) mediated chain scission[83].

In spite of the great value of sodium azide and organic azides as starting materials for synthesis of azido-functionalized polysaccharides, or as intermediates (organic azides) in alkyne-

azide cycloaddition, they are potentially explosive substances that can decompose (potentially violently in the case of small molecule azides) triggered by only slight input of energy (heat, light, and/or pressure)[84]. Besides their explosive tendencies, potential toxicity of both residual organic azides on polysaccharide backbones and azide ion should also be taken into account. Azide ion has a toxicity comparable to that of cyanide ion (LD_{50} = 27 mg/kg for rats)[85]. It becomes even worse when azide is acidified to form HN_3, which is volatile and highly toxic. From this point of view, starting materials, intermediates and products with azide residues from CuAAC should be prepared, stored, and used with caution.

Last, the 1,2,3-triazoles formed through the alkyne-azide cycloaddition can be potentially bioactive pharmacophores[86]. the bioactivity of which may become problematic if it is not the desired effect.

2.3 Metal-free [3+2] cycloaddition

As mentioned in the last section, the copper catalysts that are necessary in CuAAC are toxic and problematic in areas including polysaccharide modification, thus limiting the applications of CuAAC. For this reason, alternative means of metal-free 1,3 dipolar cycloaddition with azides have been explored, such as strain-promoted [3+2] azide-alkyne cycloaddition (SPAAC)[87], and azide-oxanorbornadiene associated cycloaddition[88].

2.3.1 Strain-promoted [3+2] azide-alkyne cycloaddition

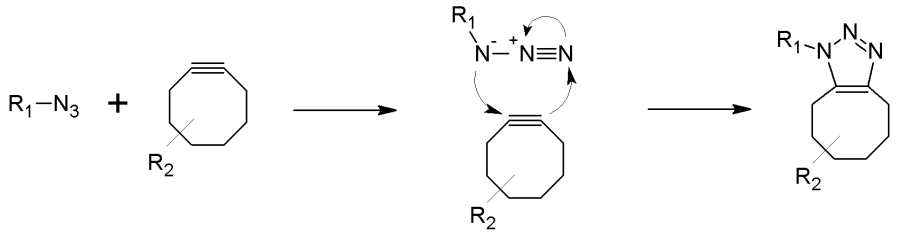


Figure 2.8. Representative SPAAC reaction between azide and cyclooctyne.

In its classic version, the azide-alkyne cycloaddition may be problematic due to slow reaction rates in the absence of elevated temperatures. A metal-free alternative is SPAAC (**Figure 2.8**), first developed by the Bertozzi group[87], that takes advantage of the ring strain of cyclooctyne as an effective way to lower the activation barrier of the cycloaddition reaction. This seminal work has been adopted rapidly by other groups as a powerful and benign coupling

strategy in polymer and material science[89,90]. Meanwhile, intensive studies dedicated to the development of cyclooctyne reagents or cyclooctyne analogs have generated more reactive activated reagents (**Figure 2.9**) by adding electron-withdrawing groups or increasing ring strain, the reaction rates of which are comparable to those of ligand-less CuAAC[21].

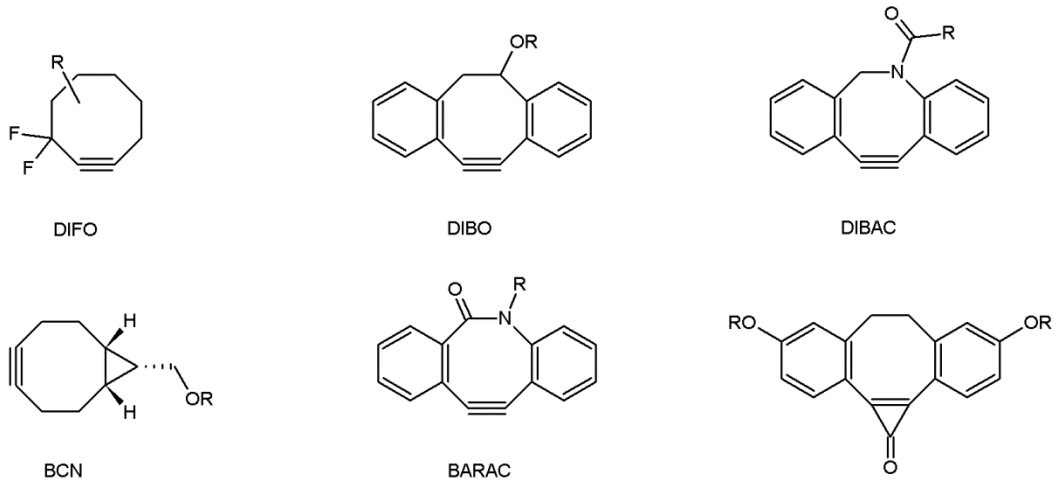
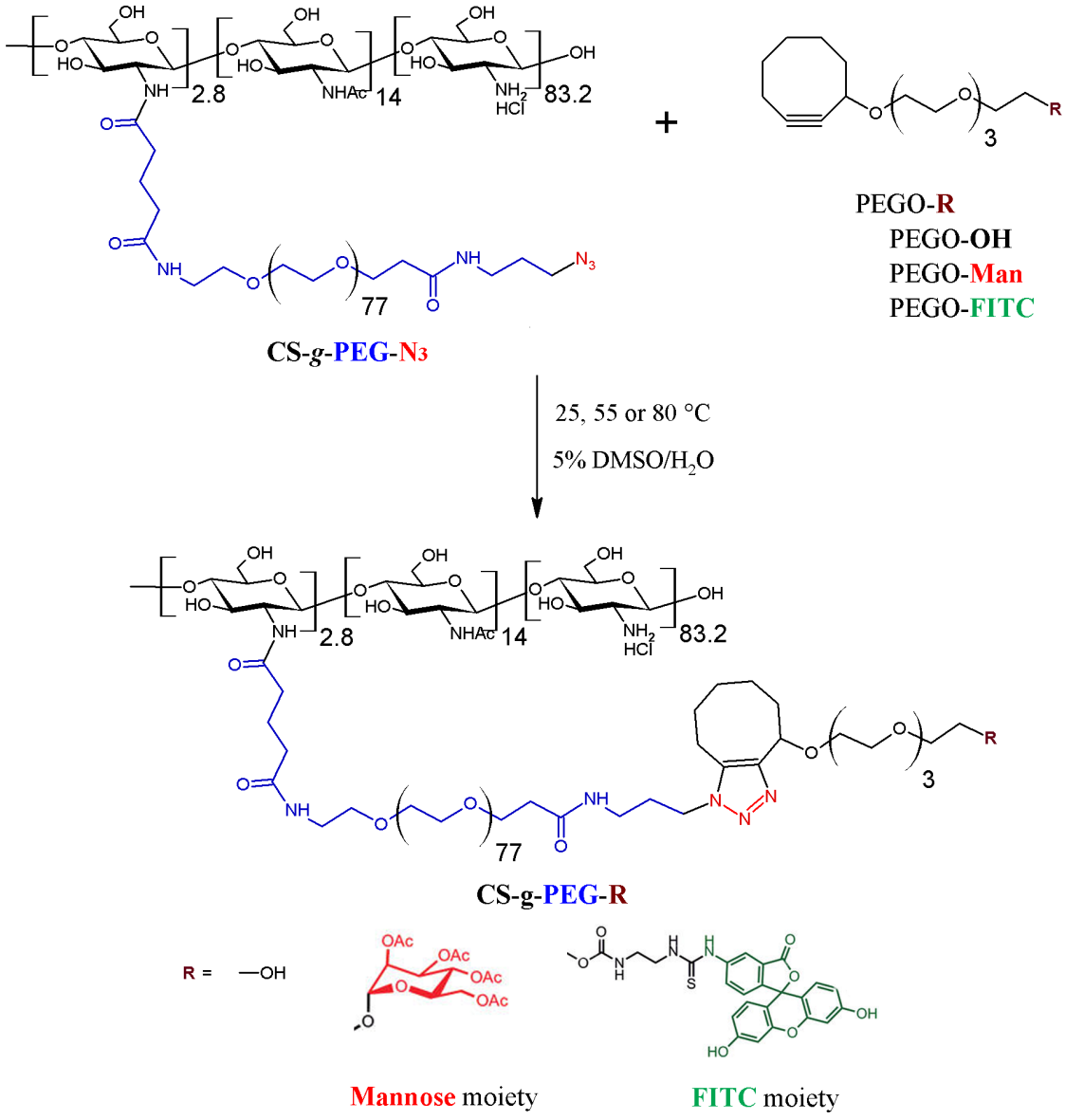


Figure 2.9. Activated cyclooctyne derivatives used in SPAAC bioconjugations. Difluorocyclooctynes (DIFO), dibenzocyclooctynes (DIBO), dibenzoazacyclooctynes (DIBAC), bicyclo[6.1.0]nonynes (BCN), and biarylazacyclooctynones (BARAC). Reproduced with permission from Ref. 21[21]. Copyright 2011, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Application of metal-free AAC to polysaccharide modification was first reported by the Fernandez-Megia and Riguera group[83]. In their research (**Figure 2.10a**), azide-terminated PEG functionalized at the other end with a carboxylic acid was grafted onto the chitosan backbone under EDC catalysis, providing a handle for subsequent click reactions. The product CS-g-PEG-N_3 was first coupled with PEG functionalized cyclooctyne (PEGO-OH) in 5% DMSO/H_2O to evaluate the conditions required for, and efficiency of the SPAAC reaction. Monitoring the progress of the click functionalization was achieved by disappearance of vicinal proton signals to the azide group. Using 2.5 equiv. cyclooctynes per 1 equiv. N_3, complete conversion was observed after 4 hours at 80 ºC, although at lower temperatures (25 and 55 ºC), the reaction took as long as 86 h for complete conversion. More importantly, while the authors observed a depolymerizing effect of Cu(I) when applying CuAAC to polysaccharides [polymer (% decrease in MW): mannan (3%), dextran (38%), chitosan (95%), and hyaluronic acid (>99%)],

depolymerization was not observed in the SPAAC reaction under similar conditions. More sophisticated cyclooctynes, i.e. mannose and FITC PEGO derivatives, were also tested and showed complete conversion with excellent mass recoveries, showing the modular profile of this reaction. Moreover, the authors extended the applicability of SPAAC to functionalizing nanoparticle surfaces (**Figure 2.10b**). CS-g-PEG-N_3 and fluorescent CS-g-PEG-FITC were crosslinked by citric acid/EDC to give nanoparticles with -N_3 handles and fluorescent markers on the surface. These nanoparticles were then successfully coupled with IgG functionalized cyclooctyne by SPAAC, further demonstrating the versatility of this chemistry.

a)


2.8
14
83.2
77
3
R
PEGO-R
PEGO-OH
PEGO-Man
PEGO-FITC
CS-g-PEG-N3
25, 55 or 80 °C
5% DMSO/H2O
CS-g-PEG-R
R = —OH
Mannose moiety
FITC moiety

b)

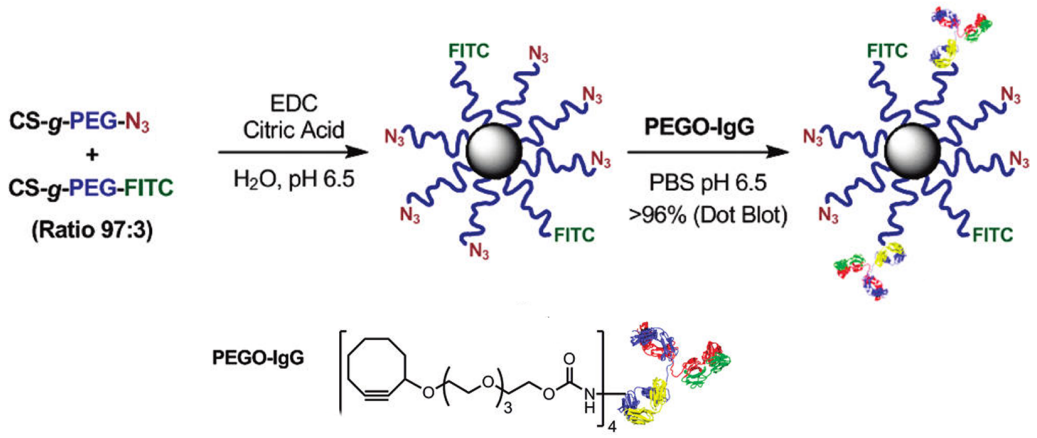

CS-g-PEG-N3
+
CS-g-PEG-FITC
(Ratio 97:3)
EDC
Citric Acid
H2O, pH 6.5
FITC
N3
PEGO-IgG
PBS pH 6.5
>96% (Dot Blot)
PEGO-IgG

Figure 2.10. a) SPAAC conjugation of CS-*g*-PEG-N_3 with cyclooctyne-functionalized PEG (PEGO-OH), cyclooctyne-functionalized PEG-Mannose (PEGO-Man), and cyclooctyne-functionalized PEG-FITC (PEGO-FITC). b) SPAAC conjugation of crosslinked CS-*g*-PEG-N_3 and CS-*g*-PEG-FITC nanoparticles with cyclooctyne-functionalized IgG (PEGO-IgG). Adapted with permission from Ref. 83[83]. Copyright 2009, American Chemical Society.

A related study was conducted by Lee et al.[91], in which ^{64}Cu was attached to chitosan nanoparticles (CNP) for an imaging application via SPAAC. In this research (**Figure 2.11**), the authors clicked azide-functionalized CNP (CNP-N_3) with a dibenzoazacyclooctyne (DIBAC) functionalized complex that was radiolabeled with ^{64}Cu. According to the authors, the copper-free click chemistry was accomplished within 30 min at 40 ºC under aqueous conditions, giving high radiolabeling efficiency and high radiolabeling yield (98%). Using the same strategy, the team further conjugated both ^{64}Cu radiolabeled complex and activatable matrix metalloproteinase-sensitive peptide (MMP-sensitive peptide) onto CNP-N_3 via a bioorthogonal click reaction, which was demonstrated to be a useful technique for cancer imaging and diagnosis[92]. In another study, instead of using azide, Jung et al.[93,94] first activated chitosan-PEG hybrid microparticle with a DIBAC handle by NHS-amine chemistry, followed by conjugation of azide-functionalized proteins and ss-DNA via SPAAC. The authors also investigated the protein conjugation kinetics, the results of which revealed multiple regimes including a rapid initial stage, an intermediate stage, and a steady and slow final stage. Hydrogel formation may also be accomplished by this chemistry. By mixing azide-functionalized and cyclooctyne-functionalized HA derivatives using a double-barreled syringe, Takahashi et al.[95] successfully obtained an *in situ* cross-linkable hydrogel under physiological conditions without the assistance of any catalyst.

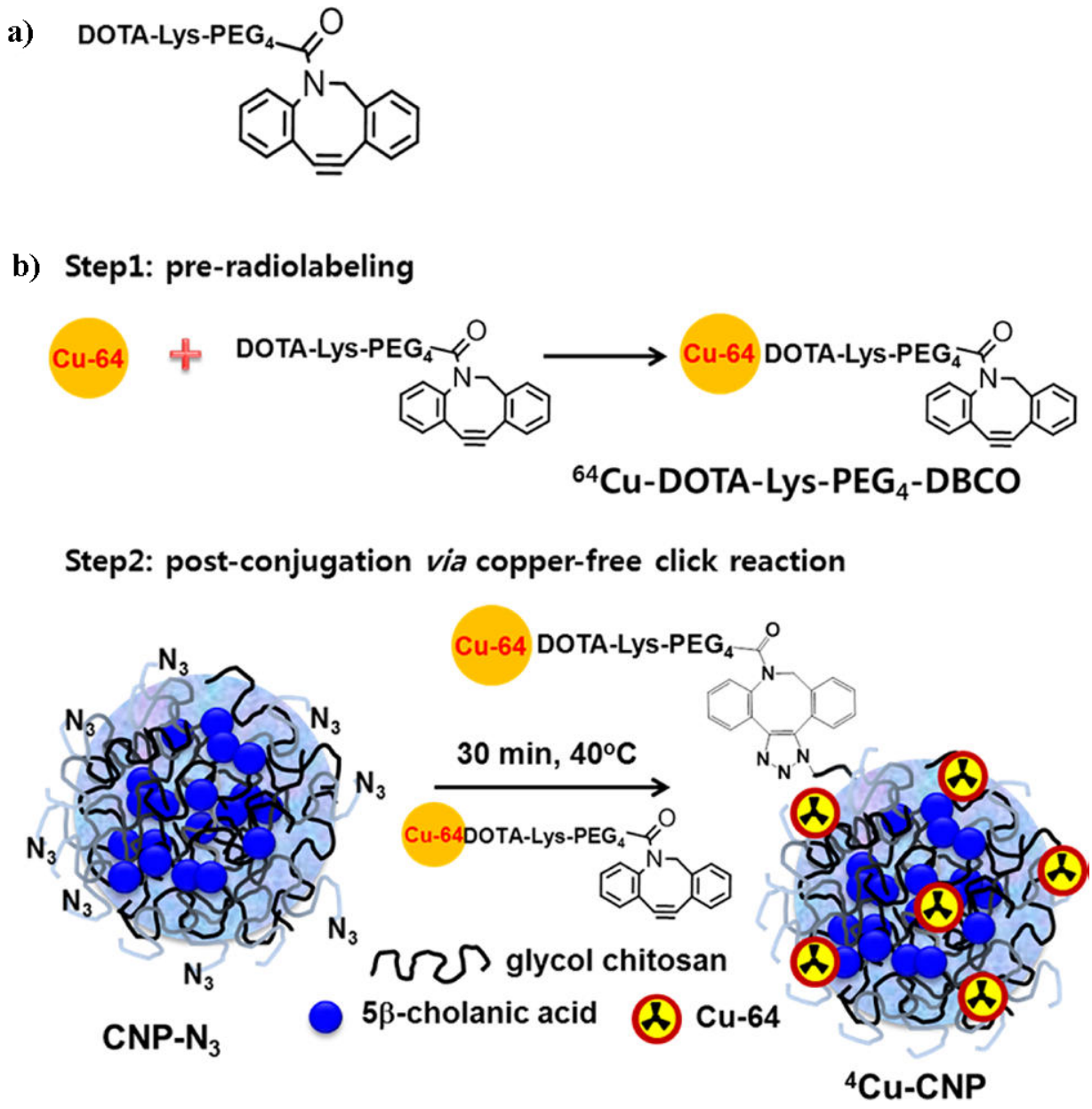


Figure 2.11. Schematic illustration for the radiolabeling of azide-functionalized CNP with pre-radio labeled DBCO-PEG_4-Lys-DOTA-^{64}Cu via copper-free click chemistry. a) Chemical structure of DBCO-PEG_4-Lys-DOTA. b) Radio labeling strategy via copper-free click chemistry: Pre-radio labeling of DBCO-PEG4-Lys-DOTA with ^{64}Cu (step 1), followed by simple radio labeling of CNP-N_3 with DBCO-PEG4-Lys-DOTA-^{64}Cu via copper-free click chemistry (step 2). Reprinted with permission from Ref. 91[91]. Copyright 2013, American Chemical Society.

Although only a handful of studies have been published using the SPAAC strategy in polysaccharide modification, they have demonstrated the high selectivity, bioorthogonality and

acceptable reactivity of this reaction under mild reaction conditions. More importantly, this approach avoids the uses of copper or other metal catalysts, which is of great value especially for biomedical applications[96]. In spite of those advantages, it is noteworthy that unlike CuAAC which gives almost exclusively 1,4-disubstituted 1,2,3-triazoles, the products of SPAAC are mixture of triazole regioisomers[83]. Other possible limitations include the lack of commercial availability, laborious lab synthesis, and instability of cyclooctynes[78]. However, as the importance of SPAAC is being proven, these issues may be successfully addressed in future work.

2.3.2 Oxanorbornadiene-azide [3+2] cycloaddition

Trifluoromethyl-substituted oxanorbornadienes are another family of reagents that can undergo [3+2] cycloaddition with azides without the use of a metal catalyst, as first reported by the Rutjes group[88] in 2007. The formation of stable 1,2,3-triazole-linked compounds is driven by an elegant tandem [3+2] cycloaddition-retro-Diels-Alder reaction, with the formation of furan as a major volatile byproduct (**Figure 2.12**). According to the authors, the oxa-bridged bicyclic systems boosted the reaction rates of the cycloaddition to approximately fivefold higher than those of the corresponding linear alkynes. Moreover, the electron-withdrawing trifluoromethyl substituent on oxanorbornadiene also contributed to a 2.3 fold increase in reaction rates for the oxanorbornadiene system. As a result, the corresponding [3+2] cycloaddition performed under ambient conditions can reach up to 80 % conversion within 800 min[88].

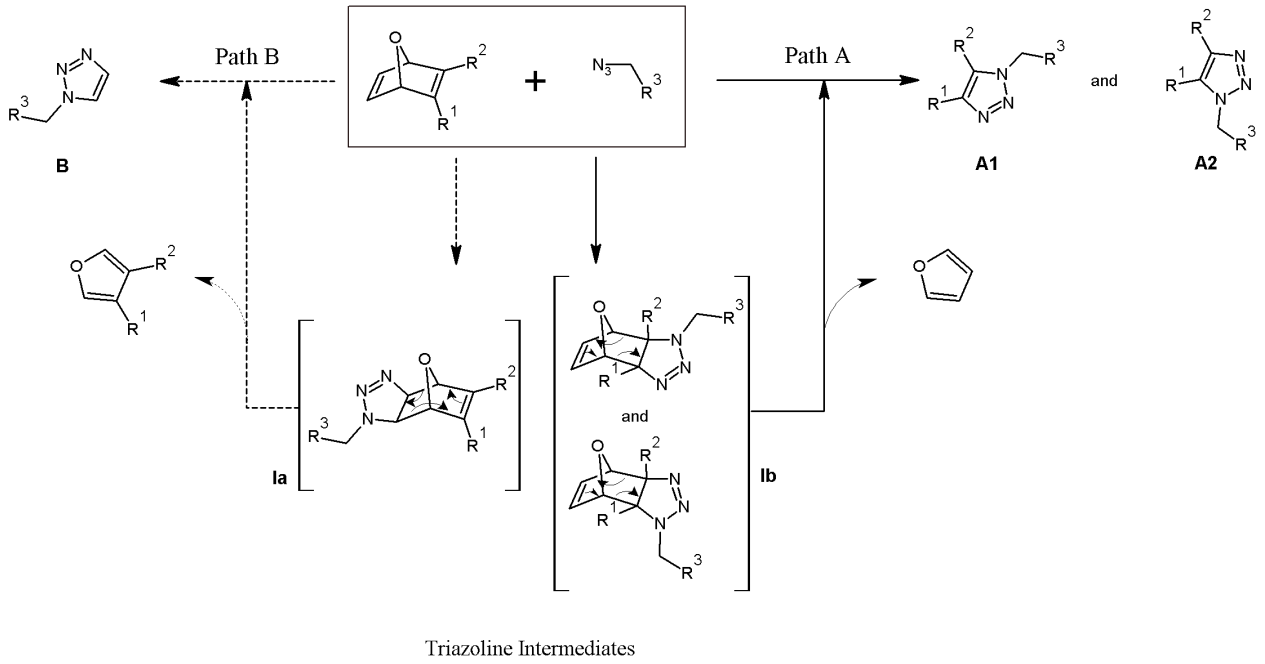


Figure 2.12. Reaction pathways of [3+2] cycloaddition of substituted oxanorbornadiene with azide for the formation of triazole compounds. Reproduced with permission from Ref. 88[88]. Copyright 2007, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

The Draeger group pioneered extension of the abovementioned reaction to the field of polysaccharide modification by conjugating cRGD-pentapeptides to oxanorbornadiene modified alginate[97]. Attachment of an ethylenediamine extended oxanorbornadiene system by amidation with the carboxylic acid groups of alginate was performed using a classical carboxyl-activation procedure with the assistance of EDC as shown in **Figure 2.13**. Bioorthogonal click reaction with different azide functionalized moieties, including cRGD-pentapeptides, was then carried out at room temperature for 4 days in aqueous media. As monitored by ^{1}H NMR as well as ^{19}F NMR (while ^{1}H NMR was sufficient to monitor the progress of cycloaddition, ^{19}F NMR was necessary to identify and quantify different isomers), almost complete transformation was observed with stoichiometric or sub-stoichiometric amounts of azide, demonstrating the modular and bioorthogonal traits of this reaction under mild conditions. Using a similar strategy, the Chirachanchai and Draeger groups also demonstrated the applicability of this reaction in the synthesis of chitosan derivatives including carboxylic acid, disulfide and silane derivatives[98], and chitosan-gold-antibody hybrids[99] in aqueous conditions.

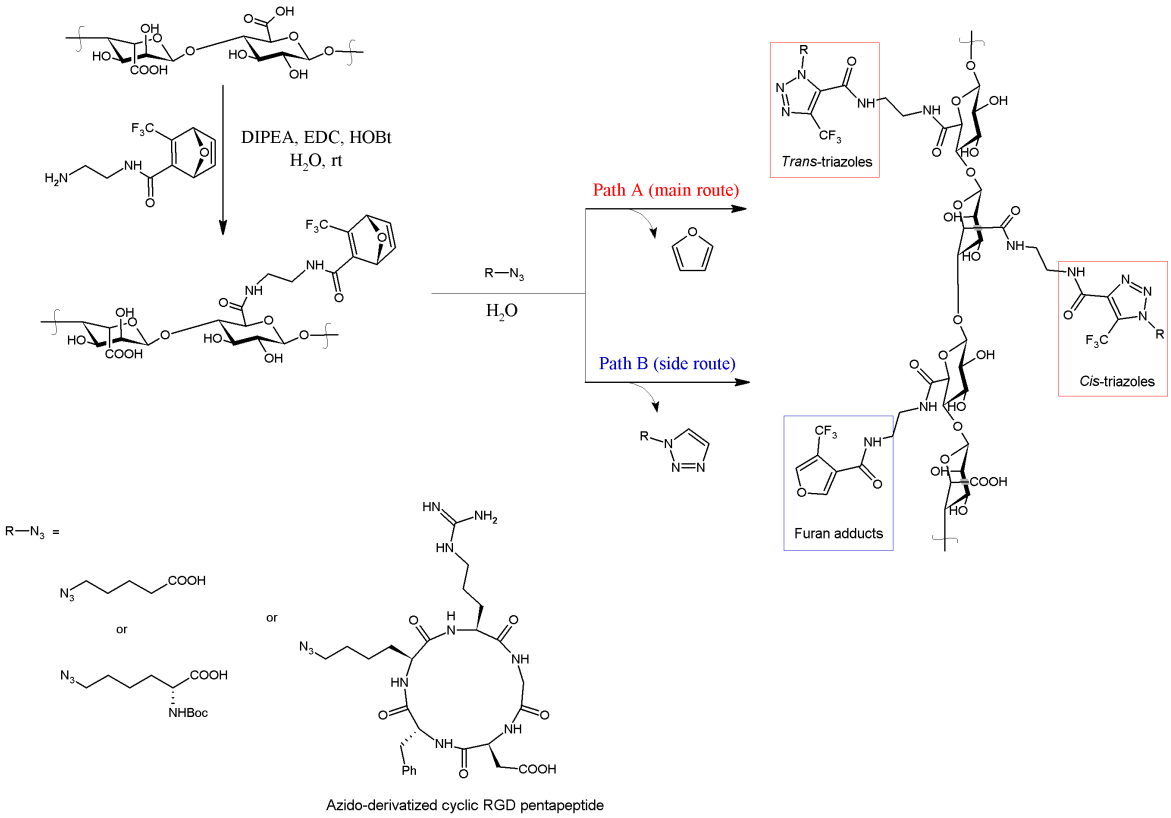


Figure 2.13. Metal-free click reaction of oxanorbornadiene-functionalized alginate with different azides. Regular cycloaddition provides two regioisomeric triazoles (path A, structures in red rectangles); undesired cycloaddition with an alternative alkene moiety results in the formation of by-products (path B, structure in blue rectangle) as also illustrated in **Figure 2.12**. Adapted with permission from Ref. 97[97]. Copyright 2012, the Royal Society of Chemistry.

Among the major limitations of this reaction are its comparatively low chemoselectivity and regiospecificity. On the one hand, both of the double bonds in the bicyclic system are able to react in the tandem [3+2] cycloaddition-retro-Diels-Alder reaction, giving both furan and triazole products, although the 1,4,5-substituted triazoles are the main products[97,98]. On the other hand, even for the 1,4,5-substituted triazoles, there are two regioisomeric forms, deriving from 1,4-cycloaddition (*trans*) and 1,5-cycloaddition (*cis*) during the click process (**Figure 2.13**). Although it has not been reported in polysaccharide chemistry, two aspects of oxanorbornadienes should be noted, i.e. their Michael acceptor profiles and retro-Diels-Alder fragmentation tendency[100]. If properly utilized, these two properties may help to generate novel reversible hydrogels or drug delivery systems. Otherwise, undesired crosslinking brought by Michael

addition between nucleophiles, e.g. –OH or $–NH_2$ on polysaccharide backbones, and the electron-deficient oxanorbornadiene double bonds may be a potential problem. Overall, neither the potential of the cyclooctyne-associated SPAAC nor the oxanorbornadiene-associated [3+2] cycloaddition have been fully explored for polysaccharide modification, and we believe substantial potential exists in these areas.

2.4 Diels-Alder reaction

The Diels-Alder (DA) reaction is a [4+2] cycloaddition reaction discovered by Otto Diels and Kurt Alder in 1928[101], for which they were awarded the Nobel Prize in 1950. For a typical DA reaction, the [4+2] cycloaddition involves a conjugated diene that is electron-rich (e.g. furan), and an electron-poor dienophile (usually an alkene, e.g. maleic acid) to form a (substituted) cyclohexene system (**Figure 2.14a**). Being modular, wide in scope, giving very high yields, and generating no or only inoffensive byproducts, this reaction qualifies as a click reaction and has been widely used not only in organic chemistry[102] but in polymer and materials science[103,104].

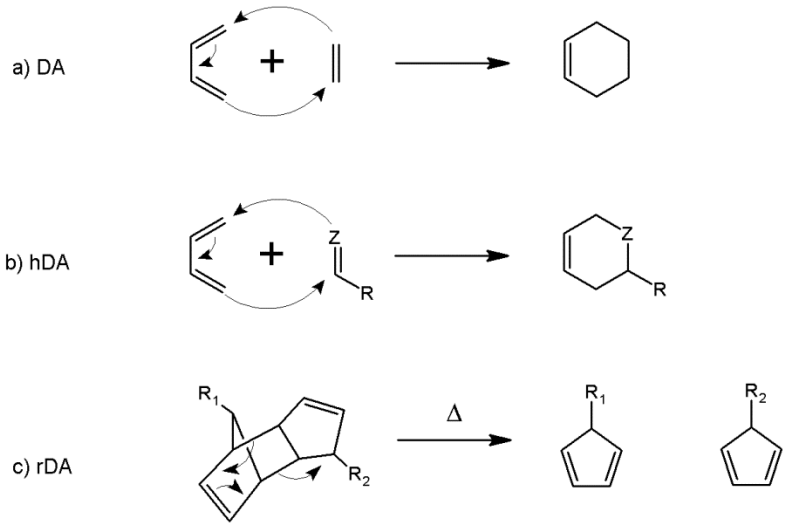


Figure 2.14. a) Diels-Alder (DA), b) hetero Diels-Alder (hDA) and c) retro Diels-Alder (rDA) reactions.

2.4.1 Classic Diels-Alder reaction

Although it has made a tremendous contribution to organic synthesis, application of the DA reaction to polysaccharide modification is relatively new. One of the most studied fields of application has been for preparation of networks (mainly hydrogels), partially due to the fact that

this orthogonal reaction can be performed under aqueous conditions at room temperature without the need for catalysts, the products of which therefore would not require extensive washing to remove residual catalysts or unreacted coupling reagents that would otherwise be present in the network. One of the first examples was reported by Nimmo et al.[105,106] in 2011. In this approach, furan-modified HA was cross-linked in a single step in 100 mM 2-(N-morpholino)-ethanesulfonic acid (MES) buffer at pH 5.5 (reaction temperature and time were not reported) via DA reaction using a bismaleimide poly(ethylene glycol) as the cross-linker (**Figure 2.15**). FTIR spectroscopy was employed to characterize the gelation by tracking the decrease of absorbances at 1455 (C=C stretch from furan), 695 (=C-H bending from maleimide) and 1466 (C=C stretch from maleimide) cm^{-1}, and the appearance of a new absorbance at 1459 cm^{-1} (C=C stretch from the DA adduct). By controlling the diene to dienophile ratio, the authors were able to tune the shear moduli of the hydrogels in the range of 100-1000 Pa, which are similar to those of brain and nerve tissues. Minimal swelling, complete degradation by hyaluronidase, and good cytocompatiblity of the gels were also observed, suggesting their potential in tissue engineering.

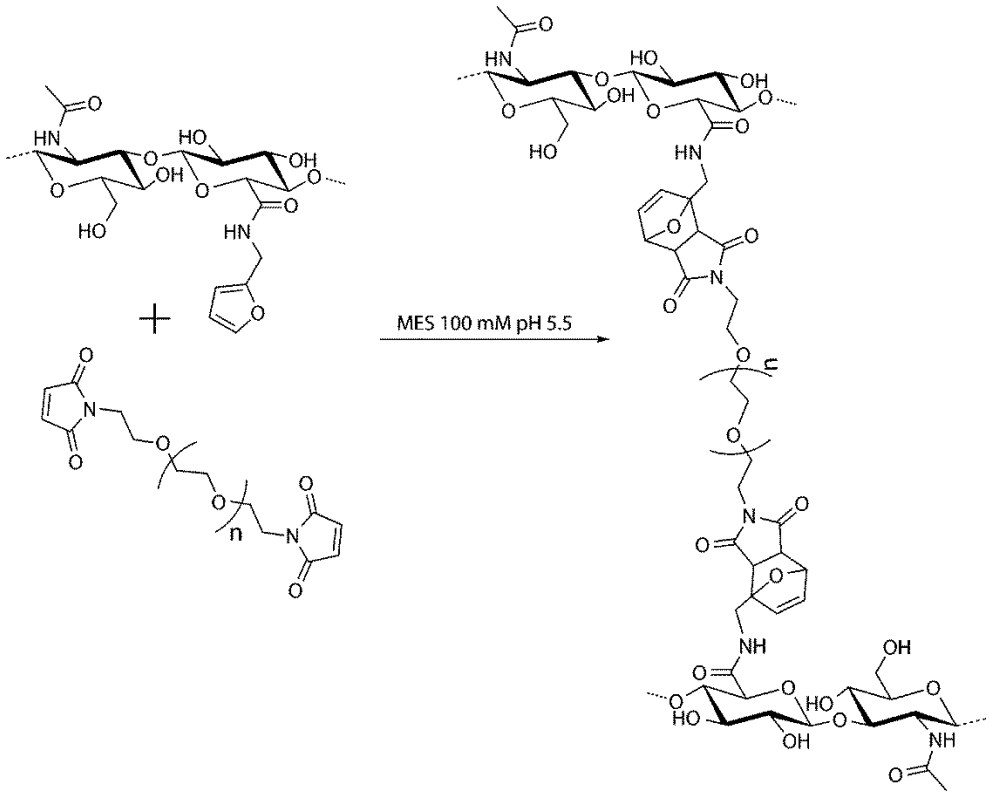


Figure 2.15. Schematic representation of formation of Diels-Alder HA-PEG hydrogels by crosslinking HA-furan with dimaleimide PEG. Reprinted with permission from Ref. 105[105]. Copyright 2011, American Chemical Society.

Almost simultaneously, a similar approach was reported by Tan et al.[107], in which a DA click reaction was performed between maleimide- and furan-functionalized hyaluronic acids in PBS and 100 mM MES buffer at 37 ºC. Gelation occurred approximately 26 min after mixing, and was complete at 40 min. With the investigation of mechanical properties, degradability, and cytocompatability, the authors also examined the potential of the hydrogel to load and release proteins, the results of which showed that the hydrogel has potential as a drug delivery system. Other investigations of similar chemistry include the synthesis of injectable HA/PEG hydrogel[108], interpenetrating HA/gelatin/chondroitin sulfate hydrogel[109], and hydroxylpropyl methylcellulose-based hydrogels[110].

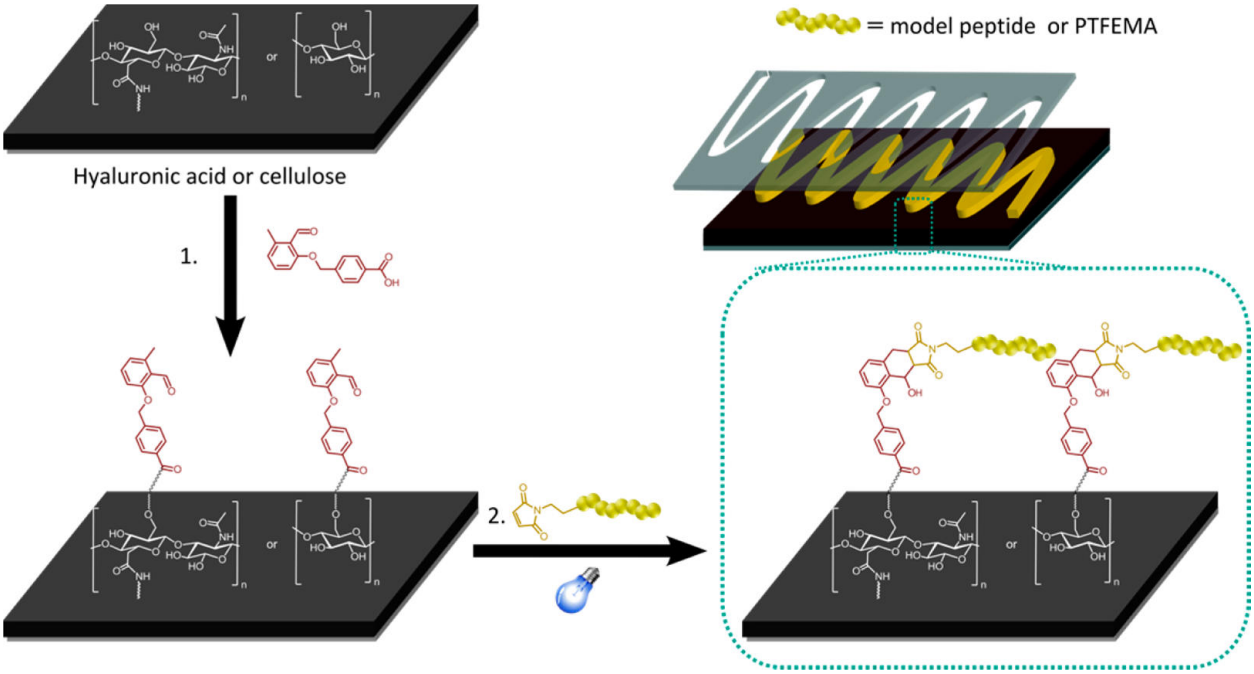


Figure 2.16. Synthetic route for spatially controlled functionalization of HA and cellulose films with tailor-made peptides or synthetic polymer strands via photoenol ligation. Reprinted with permission from Ref. 111[111]. Copyright 2013, American Chemical Society.

Indeed, the fact that the DA reaction can proceed rapidly under very mild conditions in the absence of catalyst has a number of merits in organic synthesis. However, in areas such as biosurface modification, spatial and/or temporal control of the reaction is sometimes necessary so that the reaction and thus the corresponding property changes only occur when and where a stimulus (e.g. irradiation) is applied[112]. Considering the lack of such control over the standard DA reaction, Barner-Kowollik's group demonstrated a proof-of-concept design to achieve spatiotemporally controlled functionalization of cellulose and HA surface via DA reaction[111]. In

their study, o-quinodimethane (photoenol) was functionalized onto the surfaces of cellulose and HA films and was used as a phototrigger. Upon irradiation with UV light, the photoenol underwent isomerization to its reactive diene form, which was trapped by subsequent irreversible DA reactions with a maleimide-functionalized model peptide and poly(trifluoroethylmethacrylate) (**Figure 2.16**). Successful grafting, which was confirmed by XPS and ToF-SIMS, was achieved within 2 h irradiation at 320 nm in DMF. With this technique, the authors realized patterning of peptides and synthetic polymers on polysaccharide surfaces, which opened doors to bioanalysis and other related applications.

2.4.2 Hetero Diels-Alder reaction

The dienophiles in the powerful Diels-Alder reaction are not confined to alkenes. Cycloaddition can also be initiated between a reactive diene and certain highly electron deficient heteroatom-containing double bonds (e.g. nitrosocarbonyl compounds[113], and thiocarbonyl thio compounds[114]). This reaction type, termed "hetero Diels-Alder" (hDA, **Figure 2.14b**), has also proven to be a fast and efficient click reaction. In 2009[114] the Barner-Kowollik group demonstrated an efficient, extremely rapid, room temperature conjugation strategy via hDA that utilized a cyclopentadienyl group (Cp) as the diene and thiocarbonylthio compounds as dienophiles. Later, they reported the first applications of hDA to modify cellulose surfaces. In their research, a Cp-functionalized cellulose surface was modified by a thiocarbonylthio-capped poly(isobornyl acrylate)[115] and a thioamide-functionalized peptide[116] (**Figure 2.17**). As the thiocarbonylthio compounds are also RAFT agents, this creates a modular method for surface modification of cellulose. In other words, as reactive hetero dienophiles, any RAFT generated polymers containing these thiocarbonylthio moieties can theoretically be grafted onto Cp-functionalized cellulose surfaces using this strategy. As the authors have pointed out, however, it is noteworthy that the Cp functionality has a tendency to undergo dimerization. As a result, it was recommended to perform the reaction at ambient temperature[116]. Also to be noted, a substantial amount of trifluoroacetic acid (TFA) was required in the cycloaddition as a catalyst in the RAFT-hDA process to activate the C=S double bond, when the RAFT-group bore a pyridinyl moiety[115]. Although this reaction can be performed at ambient temperature, side-reactions that TFA might introduce, e.g. esterification of TFA with free polysaccharide hydroxyl groups or acid-catalyzed polysaccharide chain scission, need to be taken into account when considering this approach to polysaccharide modification.

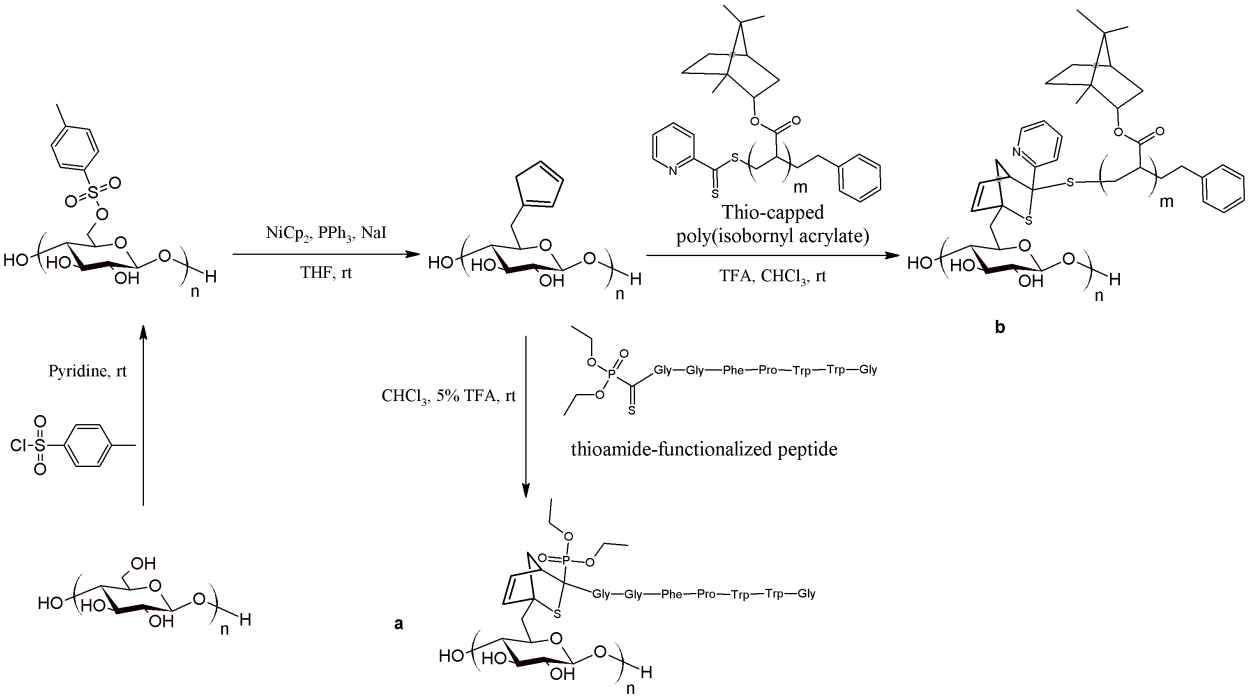


Figure 2.17. General strategy for (a) coupling of a thioamide-functionalized peptide (Phos-GGFPWWG) and cyclopentadienyl-functionalized cellulose surface; and (b) coupling of the thiocarbonyl thio-capped poly(isobornyl acrylate) and cyclopentadienyl functional cellulose surface. (a) was adapted with permission from Ref. 116[116]. Copyright 2012, Wiley-VCH Verlag GmbH. (b) was adapted with permission from Ref. 115[115]. Copyright 2011, American Chemical Society.

2.4.3 Retro Diels-Alder reaction

Another reaction (retro-DA, rDA, **Figure 2.14c**) related to the DA reaction is the decomposition of certain DA (or hDA) products. In some cases this process is thermally reversible, which is of great value in the synthesis of self-healing polymers[104]. Ax et al.[117] synthesized networks of cellulose by crosslinking furan-pendent hydroxyethylcellulose with 1,6-bis(N-maleimido)hexane (75 ºC, DMSO, 25 h). The rDA process was observed and characterized by UV spectra which indicated the restoration of the furan group when the networks were heated to 140 ºC for 21 h. The authors claimed the successful preparation of a thermoreversible network. However, data concerning the capability of the polymers to undergo further DA cycloaddition after the rDA reaction was not reported. Wei et al. conducted a more comprehensive study on dextran-based self-healing hydrogels via reversible DA reaction[118]. In this study, fulvene groups were utilized as dienes and linked to the dextran backbone by

esterification (Dex-FE), while dichloromaleic anhydride capped PEG (PEG-DiCMA) served as dienophile and cross-linker. Gelation was performed by simply mixing Dex-FE and PEG-DiCMA in PBS buffer under physiological conditions (pH 7.0, 37 ºC) (**Figure 2.18**). By varying the molar ratio of Dex-FE and PEG-DiCMA, the gelation rate as well as the morphology of the hydrogels can be controlled. Interestingly, the gels formed by the DA reaction were capable of self-healing, which was evidenced by both direct visual examination and mechanical tests. Optical microscopy results showed that self-healing of the split hydrogels achieved 98.7% after incubating at 37 ºC for 7 h. The authors rationalized the self-healing performance as being caused by dynamic equilibrium between uncoupling and recoupling of the fulvene and dichloromaleic acid groups.

The concept of reversible crosslinking in polysaccharide chemistry by either cycloaddition (e.g. dimerization of coumarin-modified polysaccharides[119,120]) or other (e.g. Schiff-base[121] or hydrazone formation[122]) methods has been of great interest. The rDA approach discussed here has provided another option with autonomous healing potential.

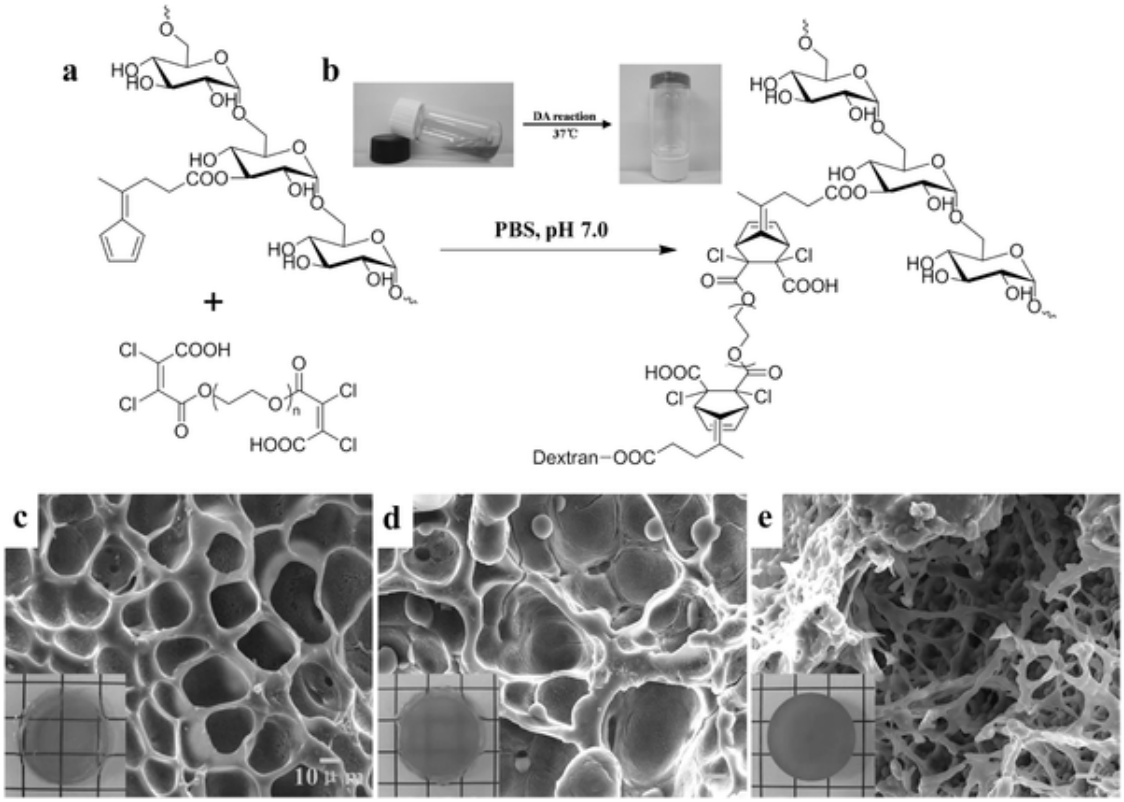


Figure 2.18. Construction strategy, SEM images, and photographs of Dex-l-PEG hydrogels ("l" means "linked by"). a) Chemical structures of the dextran-based hydrogel cross-linked by

reversible Diels–Alder reaction through the fulvene groups of Dex-FE polymeric chains and the two dichloromaleic acid groups at the end of PEG–DiCMA chains. b) Photographs before and after gelation of Dex-l-PEG hydrogel (20 wt%, R = 1) in PBS (pH 7.0) at 37 ºC. c, d, and e) SEM images and photographs of Dex-l-PEG hydrogels when R = 1, d) R = 2, and e) R = 3. Reprinted with permission from Ref. 118[118]. Copyright 2013, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

2.4.4 Inverse electron demand Diels-Alder reaction

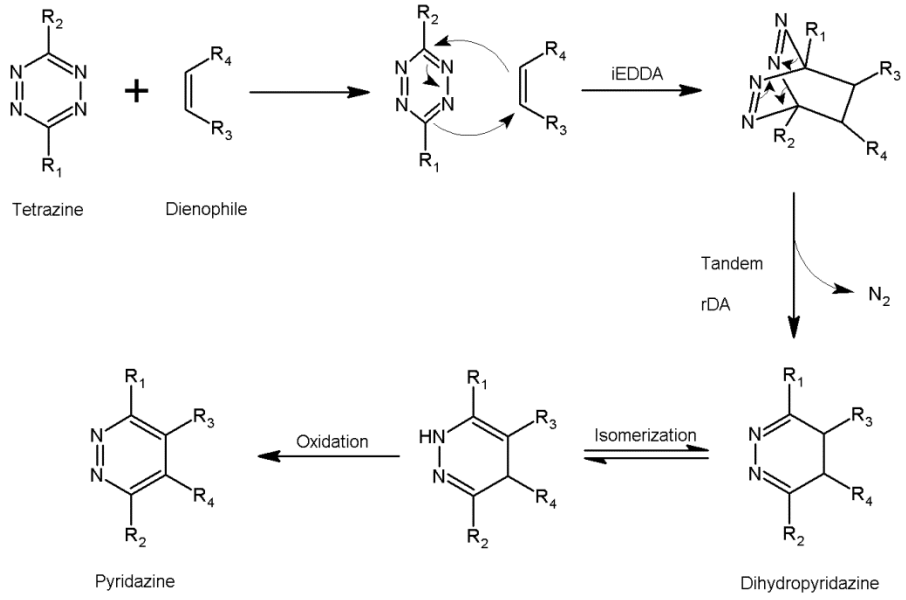


Figure 2.19. Illustration of inverse electron demand Diels-Alder (iEDDA) reaction.

Recently, a much more intriguing and promising type of DA reaction, i.e. inverse electron demand Diels-Alder (iEDDA) reaction was described almost simultaneously in 2008 by the Fox[123] and Hilderbrand groups[124] as a potential click reaction. In contrast to normal electron demand DA reactions, the tetrazine (Tz) dienes are electron deficient, and dienophiles that are electron-rich are preferred in the iEDDA reaction. In this reaction, a tetrazine and a reactive dienophile (e.g. trans-cyclooctene (TCO), norbornene and their derivatives) undergo inverse electron demand DA reaction toward a highly strained bicyclic adduct, and then tandem rDA reaction takes place to form corresponding dihydropyridazines (which might be isomerized and oxidized to pyridazines) upon release of N_2 as the only byproduct (**Figure 2.19**). Besides the non-catalyzed nature of the reaction, meaning that there are no metal or organic catalyst residues to remove from the polymeric product, one of the benchmark advantages offered by iEDDA is

the impressively high rate of reaction (orders of magnitude faster) compared to those of peer click reactions (**Table 2.2**)[125].

Table 2.2. Typical reaction rates for popular bioorthogonal ‘‘click’’ reactions. Adapted from Ref. 125[125] published by the Royal Society of Chemistry.

Reaction	**Reactants**	**Rate [$M^{-1}\cdot s^{-1}$]**
CuAAC	Terminal alkyne + azide	10^{-2}–10^{-4}
Staudinger-Bertozzi ligation	Azide + phosphine	2.2×10^{-3} [c]
Electron deficient AAC	DIFO[a] + azide	8×10^{-2} [c]
SPAAC	BARAC[b] + azide	1[c]
iEDDA	Norbornene + tetrazine	1–10[d]
iEDDA	BCN(ene)[e] + tetrazine	3.8×10^{5} [f]

[a] Difluorocyclooctyne. [b] Biarylazacyclooctynone. [c] Measured in CD3CN. [d] In H_2O–MeOH (95 : 5), 21 ºC. [e] Bicyclo[6.1.0]nonene. [f] H_2O-dioxane (90 : 10), 25 ºC.

This chemistry was rapidly adopted in polysaccharide chemistry. One intriguing study was conducted by Devaraj et al., in which tetrazine-modified dextrans were used to enable *in vivo* bioorthogonal iEDDA for applications including labeling biomolecules for tracking, and as biomarkers[126]. Although cycloaddition of Tz and TCO can proceed at a much faster rate as shown in **Table 2.2**, the efficiency of *in vivo* labeling via small molecular TCO/Tz reactions was more often than not disappointing unless an excess of one click partner was used. One primary reason for the failure of the reaction to proceed at the target site was proposed to be the pharmacokinetics of the small molecule reagents, which may be rapidly cleared by the test animals. To this end, polymer modified tetrazines (PMT) carrying imaging agents (in this study, tetrazine-functionalized dextrans) were synthesized as chaser to the initially injected TCO-antibodies (**Figure 2.20**). The results showed that while the reactivity of PMT was not significantly impeded by the conjugation to dextran backbone, PMT pharmacokinetics were significantly improved (the PMTs persisted in the blood and exhibited biexponential clearance kinetics) compared with those of the small molecule tetrazine fluorophore chaser investigated in this study. As a result, successful and efficient *in vivo* bioorthogonal TCO/Tz reactions were realized by conjugating one reaction partner, Tz in this work, to dextran to optimize the pharmacokinetics.

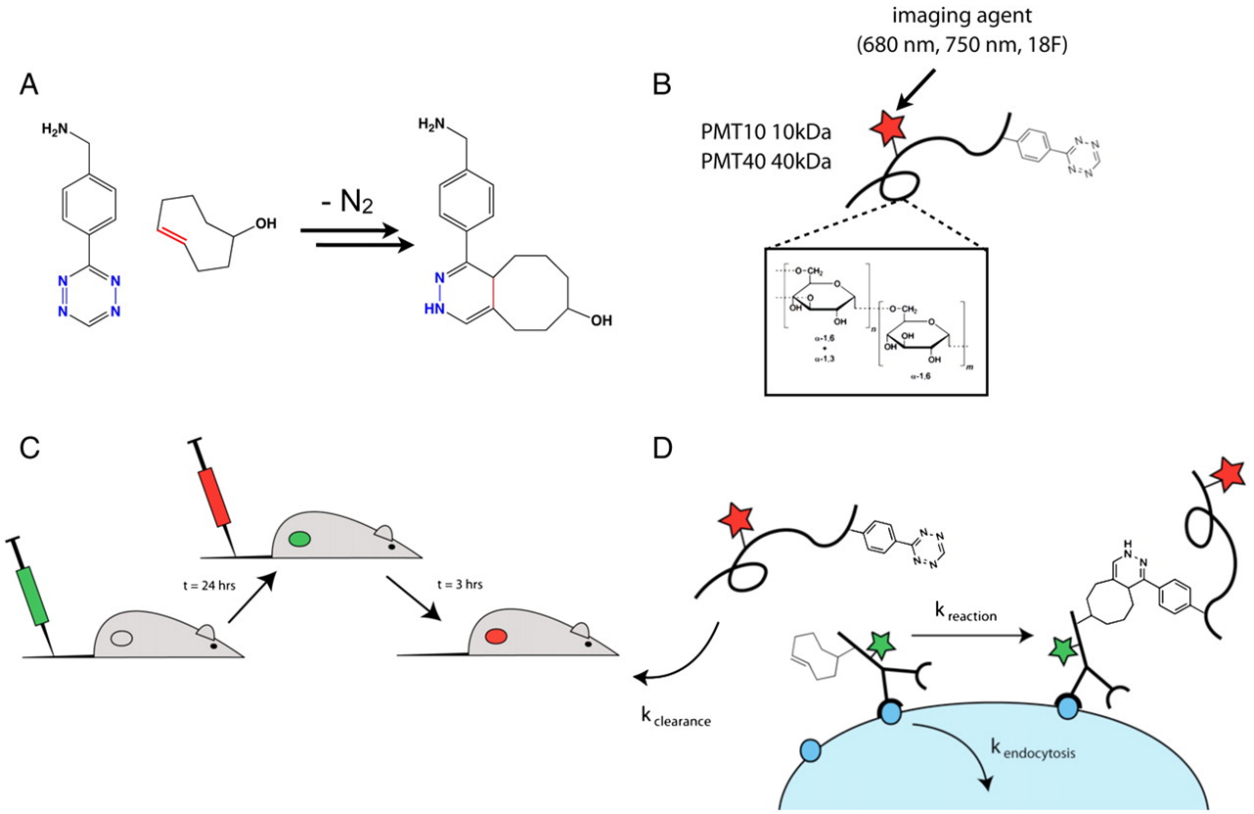


Figure 2.20. *In vivo* bioorthogonal reactions. (A) Tetrazine cycloaddition with trans-cyclooctene forming a dihydropyrazine. (B) Schematic of PMT used in this study. The scaffold consists of dextran that has been aminated to allow attachment of tetrazine reactive groups as well as imaging agents such as near-infrared fluorophores and radioisotopes. (C) *In vivo* multistep delivery of imaging agent. A slow clearing targeting agent is administered first (green) and is administered for 24 h for localization and background clearance. Next, a lower molecular weight secondary agent (red) is delivered that rapidly reacts and is cleared from the background tissue much faster than the primary agent. (D) Kinetic parameters of consideration for *in vivo* clicking. The secondary tetrazine agent reacts with transcyclooctene antibodies at a given rate ($k_{reaction}$). This rate is in competition with other rates including the clearance of the secondary agent from the body ($k_{clearance}$) and internalization of the antibody ($k_{endocytocis}$). Reprinted with permission from Ref. 126[126] published by National Academy of Sciences, USA.

Another impactful demonstration of the utility of the TCO/Tz reaction in polysaccharide chemistry was performed by the Jia and Fox groups[127]. In this work, the authors took advantage of the rapid reaction between Tz and TCO (rate constant $k_2 > 10^5$ M^{-1} s^{-1} has been measured[128])

to realize rapid diffusion-controlled interface crosslinking (**Figure 2.21**). By adding Tz-modified HA (HA-Tz) into a bath of bis-TCO crosslinker (both in water solution) via syringe, microspheres formed instantly due to fast cross-linking at the interface. Reversing the order of addition, on the other hand, afforded water-filled hydrogel channels. Moreover, the microspheres synthesized by this method exhibited excellent cytocompatibility, with the viability of prostate cancer cells encapsulated in microspheres reported as 99% and 98% at days 1 and 5 respectively.

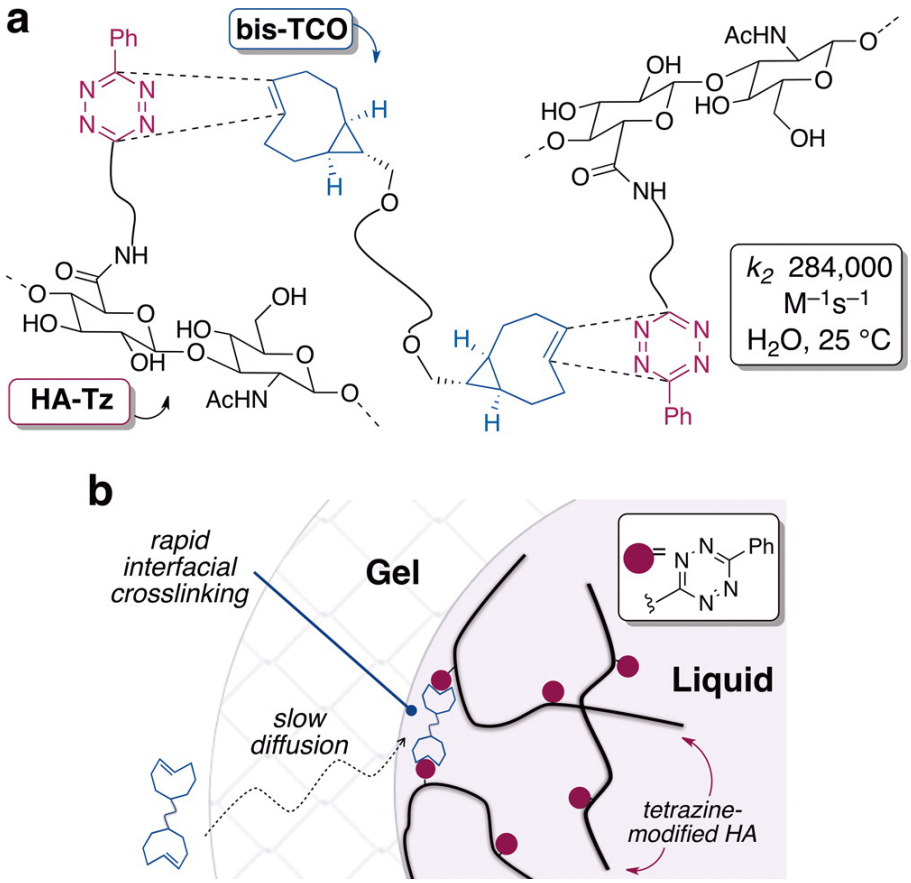


Figure 2.21. (a) Instantaneous cross-linking via tetrazine-TCO ligation. (b) Gel interface forms when a droplet of tetrazine-modified hyaluronic (HA-Tz) contacts a solution of bis-trans-cyclooctene cross-linker (bis-TCO). Cross-linking at the gel/liquid interface is faster than the rate of diffusion through the gel interface. Reprinted with permission from Ref. 127[127]. Copyright 2014, American Chemical Society.

Although there has been a surge of recent work using iEDDA reactions in polymer synthesis due to its outstanding "click" profile[125], application of this strategy to polysaccharide chemistry is still underexplored. We believe that polysaccharide chemists will soon realize the usefulness of this reaction, and employ it to generate a broad range of useful polysaccharide

derivatives. Even so, there are still several limitations that need to be taken into consideration. One major limitation is the availability of the handles (especially Tz) for "click" ligation. They are usually not widely available and oftentimes require delicate and laborious workup for synthesis[129]. Another limitation lies in the stability of strained dienophiles, such as strained TCO. During storage, these reagents need to be stored in cold solution to avoid polymerization and isomerization[130]. Further, as most current applications of this reaction have been focused on biomedical studies such as *in vivo* imaging and labeling, the bioactivities of Tz, dienophiles and their conjugation products need further exploration[131].

2.5 Thiol-Michael addition reaction and thiol-ene reaction

Michael addition is a conjugate addition reaction in which a nucleophile (Michael donor, e.g. enolate anions, –OH, $–NH_2$, or –SH) attacks the β-carbon of an α,β-unsaturated carbonyl compound (Michael acceptor) usually in the presence of a base catalyst, resulting in a new C-C, C-O, C-N, C-S or other C-X linkage. As a thermodynamically favored reaction, it fulfills some of the criteria for a "click" reaction, useful in modular and regioselective synthesis. Having a long history of application in organic and polymer synthesis[132], the Michael addition has also been used in polysaccharide modification for both discrete polysaccharide derivatives and polysaccharide networks. While carbon-based nucleophiles have been used only to a very limited extent in polysaccharide chemistry, heteroatomic donors including oxygen, nitrogen, and sulfur (oxa-, aza-, and thiol-Michael reactions respectively) have been extensively explored.

Although both oxa- and aza-Michael addition reactions in polysaccharide modification are modular under mild reaction conditions, the conversion efficiency is comparatively low[133-137]. The thiol-Michael addition to electron-deficient carbon-carbon double bonds, on the other hand, is able to achieve quantitative yields while having the attributes of other Michael reactions such as rapid reaction rates, orthogonality with other common organic reactions, and mild reaction conditions. Similar to the thiol-Michael addition, the thiol-ene reaction is another highly efficient and modular reaction that has been highlighted by Sharpless[13] and many other researchers[16,138] as a click reaction. Unlike thiol-Michael addition which features 1,4-nucleophilic conjugate addition of a thiol anion to an α,β-unsaturated carbonyl, usually in the presence of a base or nucleophile catalyst, the thiol-ene reaction is a radical based reaction in which a thiol radical, produced either by photochemical initiation or by other free-radical

initiators, attacks an electron-rich or electron-poor carbon-carbon double bond to give C-S-C linked adducts (**Figure 2.22**).

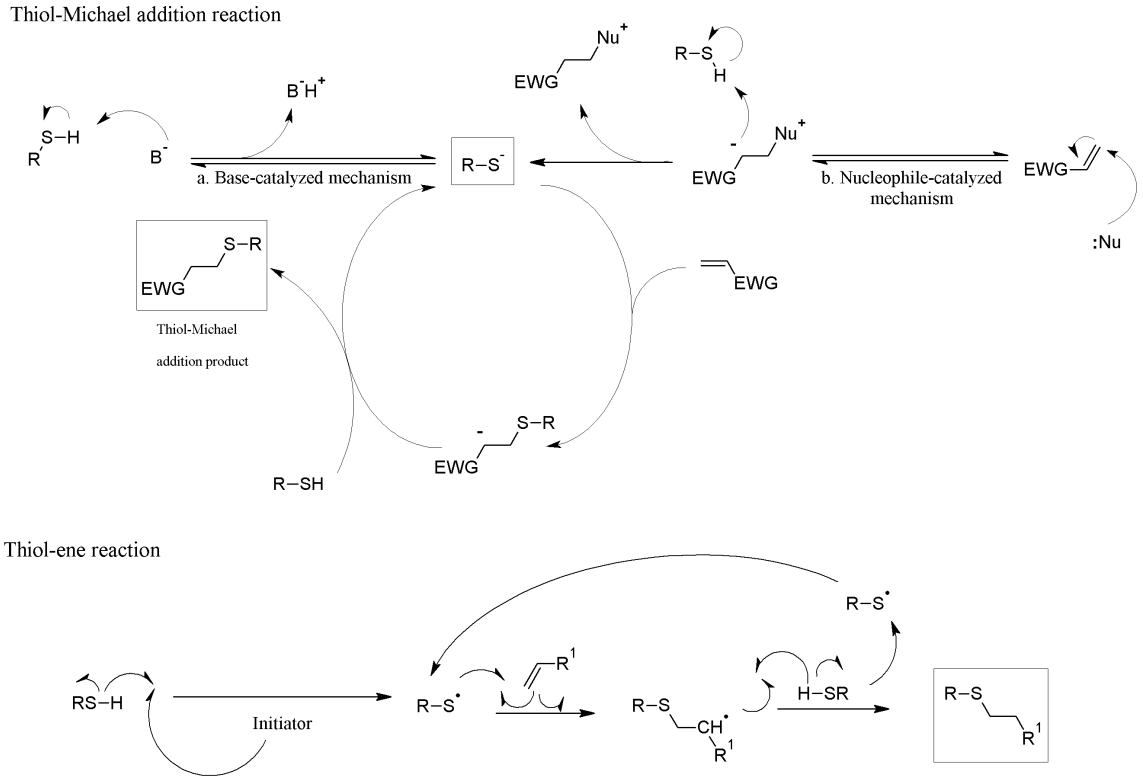


Figure 2.22. Mechanisms of thiol-Michael and thiol-ene reactions.

2.5.1 Pre-functionalization of polysaccharide backbone with olefin

To employ thiol-Michael addition or thiol-ene reactions in polysaccharide modification, it is necessary to pre-functionalize the polysaccharide backbone with either olefin (Michael acceptor) or thiol (Michael donor) functionalities as handles for the subsequent click reaction. One approach to introduce Michael acceptors to polysaccharides is through acylation. Hasegawa et al.[139] linked acrylate to pullulan derivatives using DCC as coupling agent and DMAP as catalyst in DMSO, providing a handle for thiol-Michael addition. The obtained products, which self-aggregated into nanogels in PBS buffer, were subsequently crosslinked by pentaerythritol tetra(mercaptoethyl) polyoxyethylene (PEGSH) to give raspberry-like assemblies of nanogels (**Figure 2.23**). The introduction of α,β-unsaturation could also be performed on polysaccharide surfaces. Nielson et al.[140] esterified cellulose nanocrystals with methacrylic acid in dichloromethane using the same DCC/DMAP coupling strategy. The obtained surface

methacrylated cellulose nanocrystals were then reacted with cysteamine in methanol through thiol-Michael addition, appending amino functionality that was further used as a handle for fluorescent labeling of the nanocrystals.

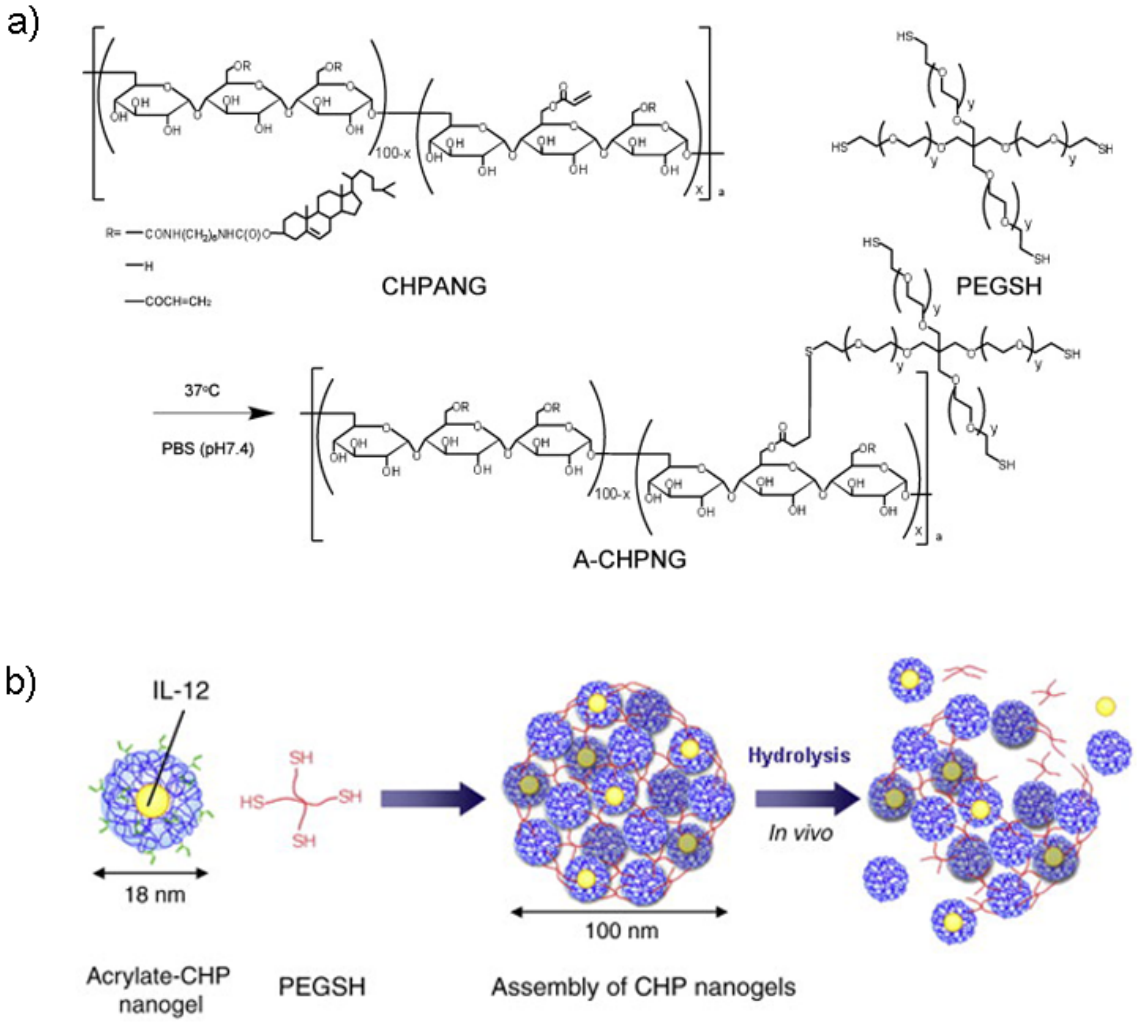


Figure 2.23. a) Illustration of crosslinking of acrylate group-modified cholesterol-bearing pullulan nanogel (CHPANG) with thiol group-modified poly (ethylene glycol) (PEGSH) by thiol-Michael addition to form raspberry-like assembly of nanogels (A-CHPNG); b) Schematic illustration of A-CHPNG formation. Adapted from Ref. 139[139] published by Elsevier.

Besides directly introducing acrylate/methacrylate onto polysaccharide backbones, spacers can be placed in between to increase the reactivity of the double bond in Michael addition by decreasing steric hindrance. For example, the Gama group employed CDI-activated

hydroxyethyl methacrylate (HEMA) to esterify mannan[141] and pullulan[142] hydroxyl groups. In the following thiol-Michael addition, a 16-carbon hydrophobic tail containing a terminal thiol group was conjugated to the appended HEMA groups under TEA catalysis in DMSO. Acrylate functionality was also introduced to the surface of cellulose nanocrystals by reacting surface hydroxyl groups with isocyanate functionalized acrylate, which then permitted the immobilization of thiolated biosensors via thiol-Michael addition in PBS[143]. It worth noting that in the synthesis/storage of acrylate/methacrylate-functionalized polysaccharides, bases that can be Michael reaction catalysts (e.g. TEA) need to be avoided to minimize undesired oxa-/aza-Michael side reactions.

Esterification[144-146] or etherification[145,147] of polysaccharides with moieties containing olefins (not in conjugation, e.g., with a carbonyl group) can provide another type of handle for radical thiol-ene reaction. Unlike conjugated olefins such as acrylates and methacrylates, those terminal olefins are more stable against undesired Michael addition. Moreover, the conversion of the coupling reaction can be easily determined by integrating the ^{1}H NMR signals of the terminal olefins, which are usually well separated from polysaccharide backbone signals. One early study was performed on solid cellulose in 2007 by Zhao et al.[145], in which long chain terminal olefins, namely 9-decenoic acid and 2-(oct-7-enyl)oxirane, were attached to the cellulose surface by esterification and etherification, respectively, under (S)-tartaric acid catalysis. The modified surfaces were readily "clickable" by photoinitiated thiol-ene reaction under UV light. This concept was then employed by Rosilo et al. for the preparation of intercalated cellulose nanocrystals and polybutadiene[146]. The cellulose nanocrystal surface was first esterified by undec-10-enoyl chloride in pyridine and DMAP to give a terminal olefin functionality. Using nonanedithiol as a crosslinker, crosslinking then occurred between the double bonds of CNC undec-10-enoate and polybutadiene by UV irradiation (DMPA photoinitiator). Auzely-Velty's group extended this concept using HA and dextran in homogeneous aqueous solutions to obtain both discrete and crosslinked products by photochemical thiol-ene reactions (**Figure 2.24**)[144]. In their study, the radical thiol-ene coupling exhibited high efficiency. Reacting at room temperature with five model mercaptans under UV irradiation (water-soluble Irgacure 2959 photoinitiator) for 5 min, the conversion of the double bonds on pentenoate-modified HA and dextran to thiol-ene adducts was up to 100% when a 3:1 feed ratio of thiol:olefin was employed.

Even a 1:1 thiol:olefin feed ratio gave conversions ranging from 70 to 95%, depending on the mercaptan used.

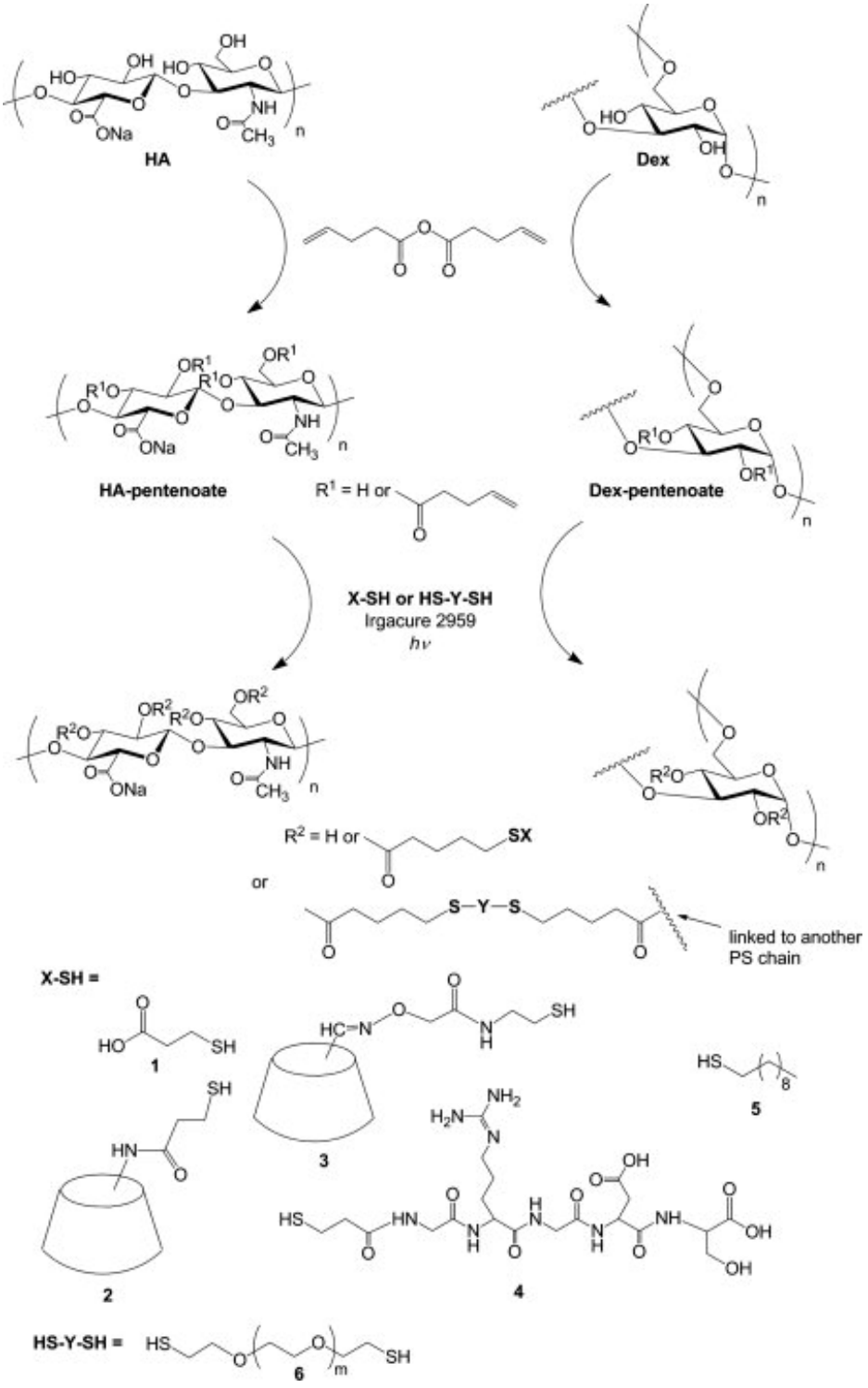


Figure 2.24. Synthetic strategy for modular functionalization of hyaluronic acid and dextran via radical thiol-ene addition. Reprinted with permission from Ref. 144[144]. Copyright 2012, Wiley Periodicals, Inc.

Not only linear alkenes, but alkenes in rings (e.g. norbornenes) can serve as thiol-ene substrates. Thiol-norbornene chemistry is such an example. Comparing with linear alkenes, strained enes like norbornenes react more rapidly with thiol radicals, but have lower reactivity toward free-radical homopolymerization due to disfavored steric effect[16]. Gramlich et al. employed this chemistry in a recent approach by esterifying hyaluronic acid tetrabutylammonium salt (HA-TBA) with 5-norbornene-2-carboxylic acid to afford norbornene-functionalized HA (NorHA), followed by subsequent reactions with di- and mono-thiols to form gels (**Figure 2.25**)[148]. In the same study, the authors also showed the potential of the photo-initiated thiol-ene chemistry for hydrogel photopatterning (**Figure 2.26**). Serving as a trigger, irradiation makes possible spatially and temporally controlled photochemistry, thus enabling photopatterned hydrogels. Although compared with the phototriggered DA reaction (**Figure 2.16**)[111] reviewed in the previous section, the involvement of photoinitiator and free-radical may limit its applications, this approach provides new understanding of the utility of photo-initiation profiles available through thiol-ene chemistry.

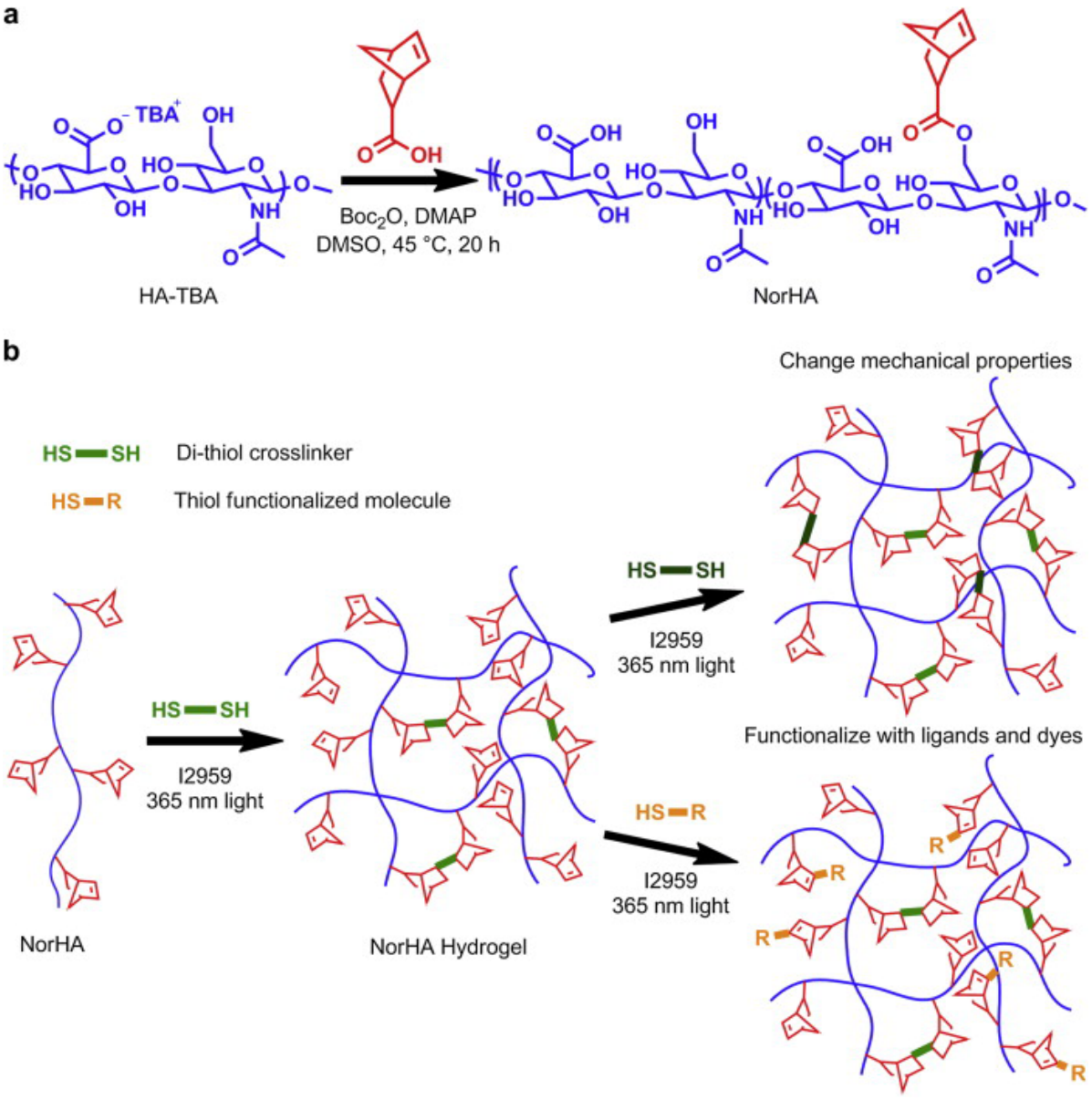


Figure 2.25. a) Scheme to synthesize NorHA from HA-TBA through the coupling of norbornene carboxylic acid to pendant alcohols on HA. b) Gels formation through the light initiated thiol-ene reaction between a di-thiol and NorHA with subsequent chemical modification with mono- and/or di-thiols. Reproduced from Ref. 148[148] published by Elsevier.

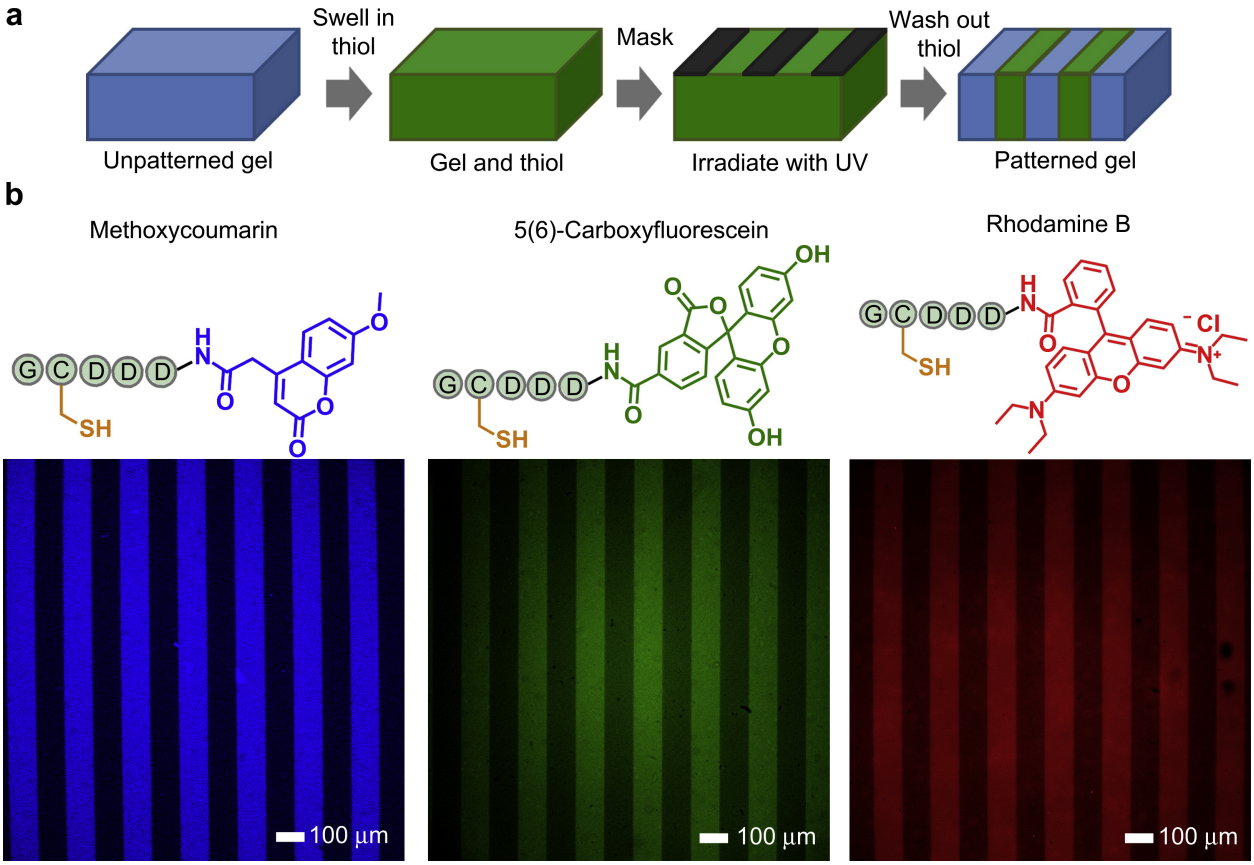


Figure 2.26. a) Schematic illustration of process to photopattern NorHA gels with thiol containing molecules; b) Confocal images of photopatterned (mask with 100 mm stripes) NorHA gels with fluorescent dye terminated peptides. Reproduced from Ref. [148] published by Elsevier.

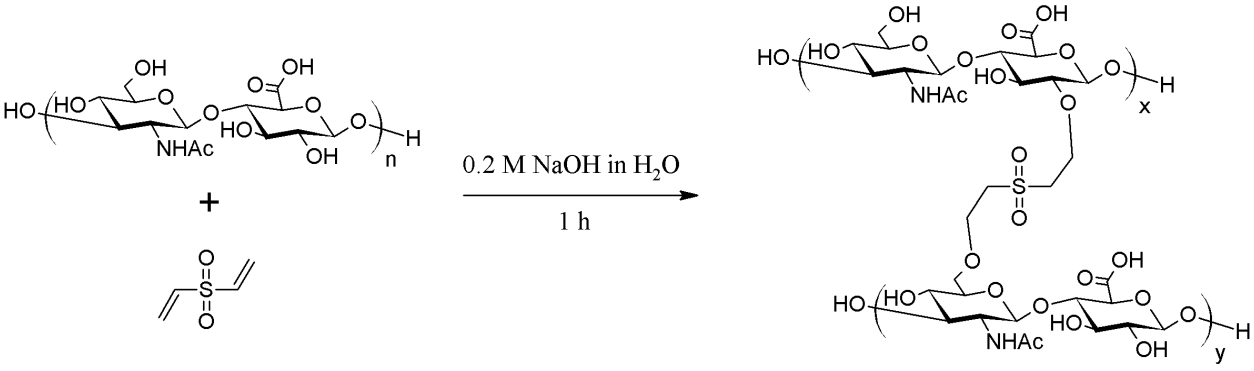


Figure 2.27. Exemplary crosslinking reaction of DVS with polysaccharide (HA). Adapted with permission from Ref. 149[149].

Vinyl sulfone (VS) is another good Michael acceptor for thiol-Michael addition. However, reacting divinyl sulfone (DVS) with polysaccharides usually leads to crosslinked

products due to nucleophilic hydroxyl/amino groups attacking the electrophilic double bond of DVS by Michael-type addition[149] (**Figure 2.27**). To obtain discrete vinyl sulfone modified polysaccharide derivatives useful as handles for subsequent thiol-Michael/thiol-ene click reactions, Hiemstra et al.[150] first carried out Michael addition of mercaptoalkanoic acids to one double bond of DVS. The product VS thioalkanoic acids were then esterified with dextran using DCC coupling agent. The VS-pendent dextran was further crosslinked by thiol-Michael addition of a multifunctional thiolated PEG (**Figure 2.28**). In another study, Yu et al.[151] reasoned that the previous crosslinking issues observed when reacting polysaccharides and DVS was due to the low molar ratios of DVS/polysaccharide used. The authors then successfully avoided crosslinking and obtained VS-functionalized water-soluble derivatives of dextran, alginate and HA by simply adding a large molar excess of DVS to the aqueous polysaccharide solutions. These VS-functionalized polysaccharides obtained by the simple one-step click reaction were capable to react with thiols at neutral pH in aqueous media. In a different approach, ene-functionalized cellulose surfaces were prepared by silylation of cellulose hydroxyls with vinyltrimethoxysilane, followed by thiol-ene click reaction under UV irradiation[152].

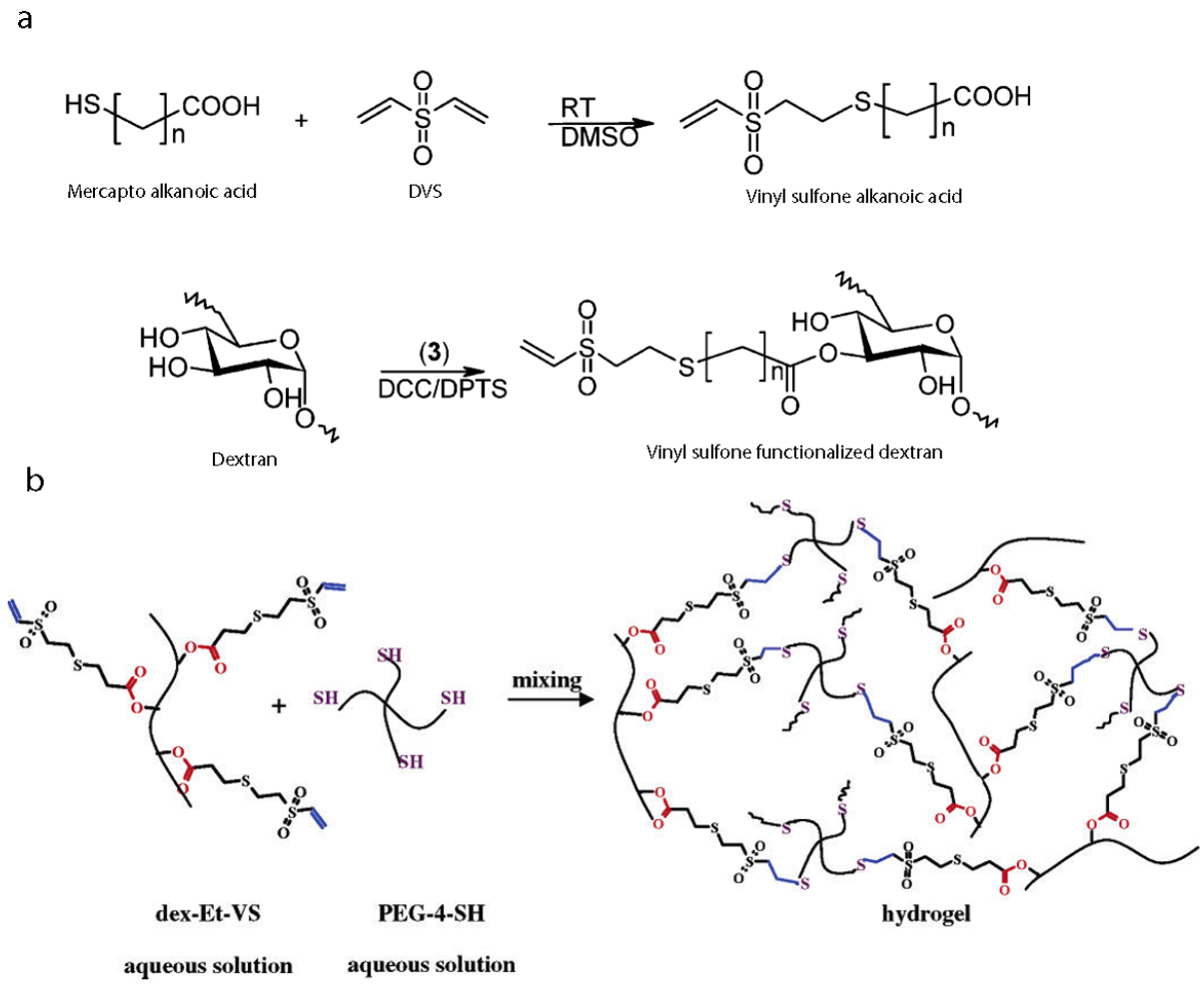


Figure 2.28. a) Schematic representation of one-pot synthesis of dextran vinyl sulfone conjugates; b) Schematic representation of Michael addition between VS functionalized dextran and multifunctional thiolated PEG. Reprinted with permission from Ref. 150[150]. Copyright 2007, American Chemical Society.

2.5.2 Thiolation of polysaccharide backbone

Besides introducing acceptor groups (e.g., carbon-carbon double bonds) to polysaccharides, adding donor groups is an alternative approach. Attaching thiol groups to polysaccharides may also render polysaccharide derivatives "clickable" via thiol-Michael/thiol-ene addition. Different approaches have been used to obtain thiol-functionalized polysaccharides. Acylation with the assistance of coupling reagents is one of the most common approaches. Wang et al. reacted N-acetyl-L-cysteine (NAc) with chitosan using 1-ethyl-3-(3-dimethylaminopropyl-

carbodiimide) hydrochloride (EDAC·HCl) and 1-hydroxybenzotriazole (HOBt) as coupling reagents in aqueous media[153]. The NAc-chitosan was then incubated with maleic acid-grafted dextran to form in situ hydrogels via Michael type thiol-ene reaction[154]. For polysaccharides containing carboxylic acids, e.g. HA, the carboxylic acid can be activated by EDC and then reacted with cystamine, giving regioselectively thiolated derivatives[155]. However, the appended, unprotected thiol group is reactive and likely to be esterified in the presence of coupling reagents. To minimize this possibility, disulfides were used. For example, the thiolation can be accomplished by reacting chitosan derivatives with 3,3'-dithiodipropionic acid (DTP) in the presence of coupling reagents (e.g. EDC and NHS), followed by the addition of 1,4-dithio-DL-threitol (DTT) to reduce the disulfide bonds to thiols[156]. A similar strategy was used for synthesis of thiolated HA derivatives[157,158].

Other thiolation methods such as silylation with thio-pendent silyl moieties[152] have also been reported. Among them, direct thiolation of the polysaccharide backbone was intriguing, for example as described by the Kros group. Thiolated β-cyclodextrin (β-CD-$(SH)_7$) was synthesized via a two-step reaction. First, the primary hydroxyl group of β-CD was selectively transformed to iodide using PPh_3/I_2. The iodide was subsequently converted to thiol in a one-pot reaction with thiourea (**Figure 2.29**). The resulting β-CD-$(SH)_7$ was utilized as a platform for thiol-ene/Michael type additions in a variety of interesting applications including formation of CD-dextran for delivering hydrophobic drugs[159,160] and synthesis of CD-centered star polymers[161]. Admittedly, β-CD is an oligosaccharide rather than a polysaccharide. Nevertheless, this synthetic pathway is likely to be applicable to many polysaccharides which have primary hydroxyl groups (e.g. cellulose[33], curdlan[162], pullulan[162,163]), although, to the best of our knowledge no such study has been performed on polysaccharides.

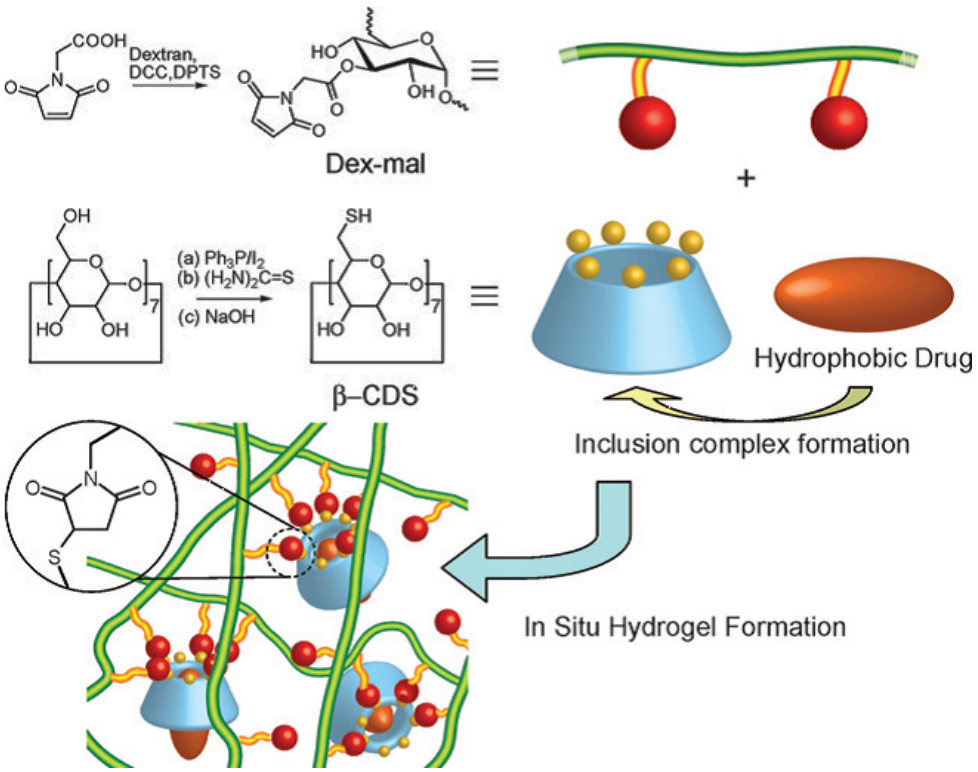


Figure 2.29. Schematic representation of the *in situ* hydrogel forming system in which β-CDS plays two roles: dextran cross-linker and host for hydrophobic drug. Inclusion complex formation can increase the affinity of the hydrogel for hydrophobic drugs and can prevent drug aggregation. Reproduced with permission from Ref. 159[159]. Copyright 2010, the Royal Society of Chemistry.

2.5.3 Limitations

A drawback to these reactions is the fact that thiol groups are easily oxidized and form disulfide bonds. This problem is more significant for large molecule thiol-containing reagents such as thiolated PEG and thiolated polysaccharides. Several groups have observed this type of oxidation-induced crosslinking[152,161]. As a result, careful attention needs to be paid to the preparation and storage of thiolated polysaccharides. For many small thiols, unpleasant smell as well as toxicity concerns[164] may be the major concerns preventing large scale industrial application of these reactions.

2.6 Oxime click chemistry

Oxime click chemistry, the formation of an oxime bond by reaction between an aminooxy group and an aldehyde or ketone, has emerged as a robust strategy in areas such as bioconjugation[165]. Compared with primary amines, reactivity of hydroxylamine or alkoxylamine (aminooxy) groups towards carbonyls is significantly higher[166] and the formed oximes are much more stable against hydrolysis than corresponding imines[167]. The reaction can be carried out in aqueous media under mild reaction conditions without the use of metal catalysts. Taking advantage of the fact that aldehyde functionality is available in equilibrium at the reducing end of most polysaccharides, Novoa-Carballal and Muller pioneered the synthesis of polysaccharide-b-PEG block copolymers using oxime click chemistry in 2012 (**Figure 2.30**)[168]. In their research, three unmodified polysaccharides, namely dextran, chitosan and HA, were coupled with aminooxy-functionalized PEG (MeO-PEG-ONH_2). The authors investigated the reaction conditions and found that using DMSO/pH 3 buffer (citric acid based) solution at 45 ºC gave fast and efficient reactions. Under these conditions, using 5 equiv of MeO-PEG-ONH_2 for 24 h resulted in 99% conversion to coupled block copolymers. This strategy for polysaccharide end-functionalization has been quickly adopted to obtain a variety of block copolymers such as dextran-b-PDMAEMA[169], and glycosaminoglycan-b-PEG[170], as well as end-labeled polysaccharides such as fluorophore-labeled N,N,N-trimethyl chitosan[171].

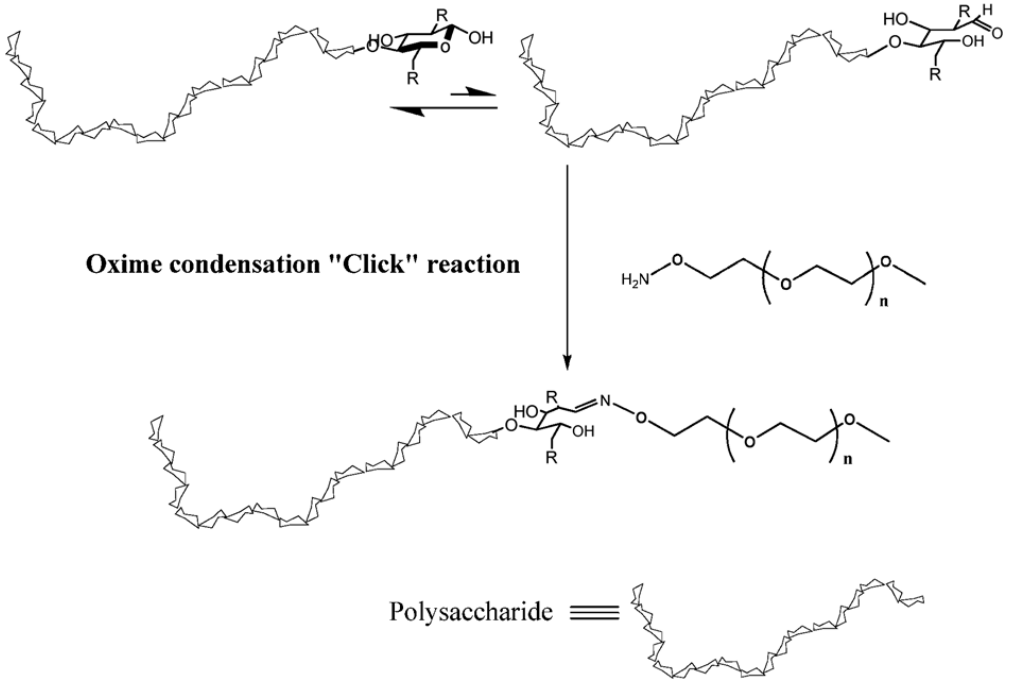


Figure 2.30. Oxime approach for polysaccharide-b-PEG synthesis. Polysaccharides used in the reference include dextran, chitosan and HA. Reproduced with permission from Ref. 168[168]. Copyright 2012, the Royal Society of Chemistry.

Sestak et al.[172] performed the oxime click reaction under similar reaction conditions: 5 equivalents aminooxy reagent (for aminooxy peptides, the ratio was 1:1) were mixed with HA in acetate buffer solution at pH 5.5 for 16 h at room temperature, followed by dialysis to remove unreacted aminooxy species (**Figure 2.31**). However, the FTIR and NMR data run counter to the assumption that coupling proceeds primarily at the reducing end of polysaccharides. Unmodified HA contains two carbonyl functional groups, i.e. a carboxylic acid and an acetamide, on each repeating unit, both of which may serve as possible handles for the oxime click reaction. In this study, the authors claimed that the oxime-bond formed at all three carbonyl carbon centers, and primarily at the carboxylic acid site. Graft efficiencies using this reaction can reach up to 90%. The authors did not state very clearly the exact meaning of the ratio (whether it was mol of aminooxy agent to HA repeating unit or to carbonyl group). However, in either condition the authors in this study employed a large excess of oxime agents compared with the 5 mol per HA molecule ratio in the previous study[168]. Moreover, from signal intensity in the provided NMR spectra of the oxime click products (HA grafted with O-carboxymethyl hydroxylamine, and HA grafted with O-benzyl hydroxylamine), the DS of the grafted functionality did not seem to be as high as stated by the authors, though the integration values of each peak required for calculating DS were not provided. From this point of view, although this research has provided a new possibility for grafting functionality on polysaccharides containing carbonyl groups such as HA and alginate, further synthetic study is needed to verify this approach.

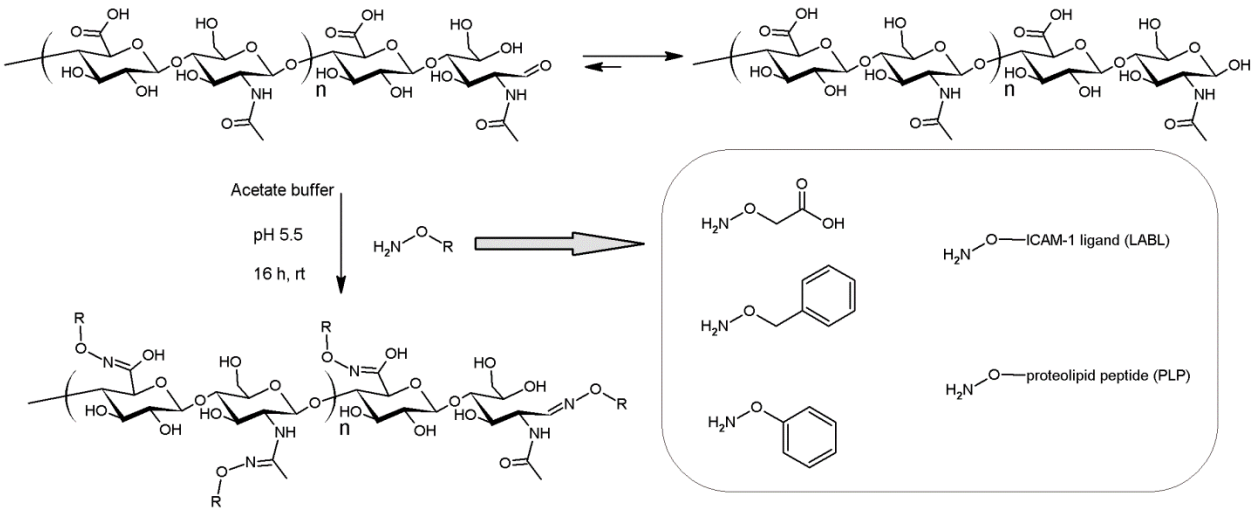


Figure 2.31. Reaction scheme of single-step grafting of small molecules and peptides to hyaluronic acid via oxime click chemistry. Adapted from Ref. 172[172] published by Elsevier.

Oxime bonds are known to be labile under acidic (pH < 2) or basic (pH > 9) conditions[168,172]. Although this lability may cause problems in synthesis, processing, and storage of these polymers, it is of interest in areas such as controlled drug release.

2.7 Olefin cross-metathesis

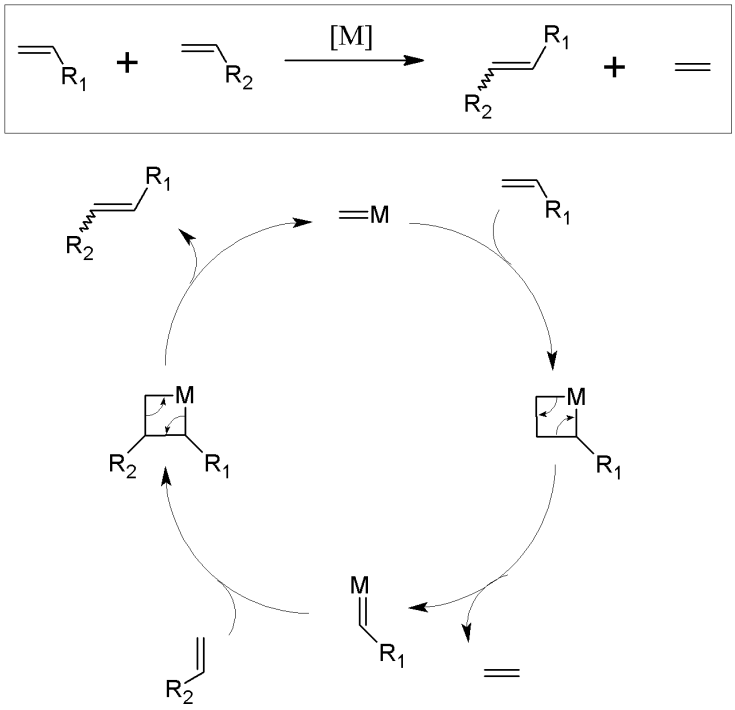


Figure 2.32. Representative olefin cross-metathesis and reaction mechanism.

Olefin metathesis has been developed in recent years as a powerful, versatile tool in organic chemistry[173]. In polymer synthesis, olefin metathesis techniques such as ring opening metathesis polymerization (ROMP)[174] have been comprehensively investigated in the past decade and have helped to generate a variety of novel polymers. Applications of olefin cross-metathesis (CM, **Figure 2.32**) to the synthesis of complex small molecules[20,175,176] including carbohydrates[177-179] have been extensively studied in the recent years thanks to the publication of Grubbs' model of selectivity for CM[20], and the development of active and selective catalysts for CM[180-182]. Based on chemical structure and reactivity results, Grubbs empirically classified olefins into 4 types (**Table 2.3**)[20]. Type II and III olefins (usually sterically-hindered and/or electron-deficient, also depending on the catalyst used) have low olefin metathesis reactivity and only slowly homodimerize, while more reactive terminal olefins (type I) readily undergo homodimerization via olefin metathesis. Moreover, the homodimers of the terminal olefins are susceptible to subsequent secondary CM reactions. As a result, when a type I olefin is reacted with a type II or III olefin, high conversion to a CM product can be achieved by employing an excess of the type II or III olefin[175].

Table 2.3. Grubbs' categorization of olefins and rules for selectivity. Adapted with permission from Ref. 19[19]. Copyright 2014, American Chemical Society.

Grubbs' Rules:

Reactions between two olefins of Type I = Statistical CM

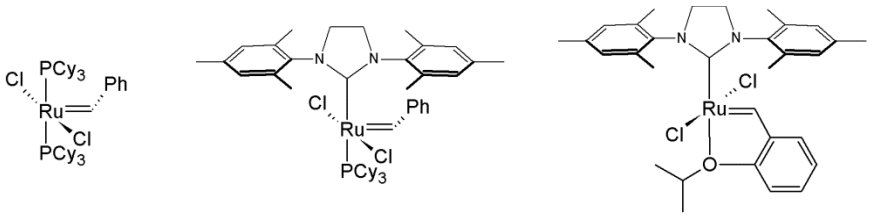
Reactions between two olefins of same type (non-Type I) = Non-selective CM

Reactions between two different types (except Type IV) = Selective CM

Olefin Type	Olefin Metathesis Reactivity	Examples[a]
Type I	Rapid homodimerization, homodimer consumable	terminal olefins
Type II	Slow homodimerization, homodimer sparingly consumable	acrylates, acrylic acids, acrylamides
Type III	No homodimerization	3º allylic alcohol (protected)
Type IV	Olefins inert to CM, but do not deactivate the catalyst (spectator)	vinyl nitro olefins

[a]Selectivity depends on catalyst used. The examples shown are valid for Grubbs' 2nd generation catalyst.

Taking advantage of the reactivity differences between the four types of CM partners, it is theoretically possible to perform a "click-like" reaction by reacting one type of CM partner with a polymer possessing a substituent containing a different type of olefin. Nevertheless, CM as a modular and mild approach to polymer modification of polymers has been little studied. One major barrier is the potential for homodimerization (via self-metathesis (SM)) of the CM partners, especially for type I olefins. In a typical example, Joly et al.[183] observed SM of cellulose undec-10-enoate using Grubbs' catalyst (1st generation, **Figure 2.33**), affording crosslinked and insoluble cellulose plastic films. For polymer modification targeting discrete and soluble products though, such crosslinking is not only undesired, but even disastrous. This problem was addressed by Meier's group in the side-chain modification of polyesters and poly(2-oxazoline)s with terminal double bonds in the side chains via CM[184,185]. In their work, polymers with terminally unsaturated side-chains (type I olefins) were reacted with type II or III CM partners including a variety of acrylates, using Hoveyda-Grubbs 2nd generation catalyst. The same group further expanded the concept to the synthesis of divergent dendrimers using the CM reaction[186].

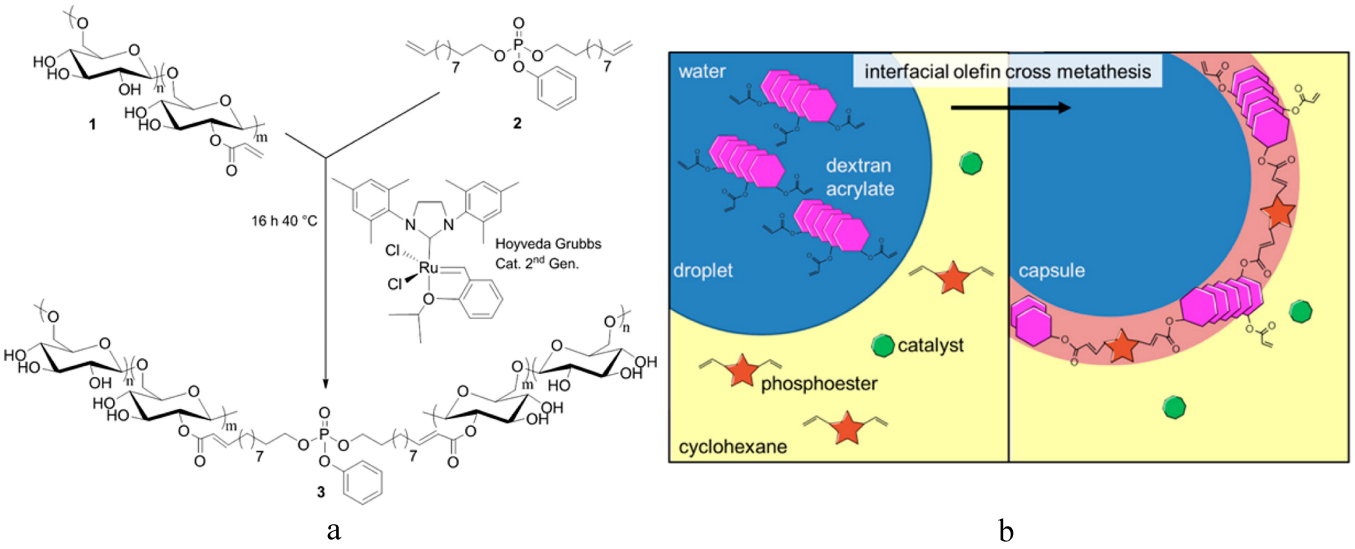


C1 C2 C3

Figure 2.33. Commonly used Grubbs' olefin metathesis catalysts; C1 Grubbs's catalyst, 1st generation; C2 Grubbs' catalyst, 2nd generation; C3 Hoveyda-Grubbs' catalyst, 2nd generation. Reprinted with permission form Ref. 19[19]. Copyright 2014, American Chemical Society.

Malzahn and coworkers reported interfacial olefin cross-metathesis of dextran acrylate with a terminally unsaturated organophosphate in an inverse miniemulsion to afford hollow nanocapsules as shown in **Figure 2.34**[187]. The authors demonstrated that the CM reaction occurred selectively at the oil-water interface of the aqueous nanodroplets. This strategy of using olefin metathesis to prepare cross-linked polysaccharides is not new (as mentioned in previous paragraph, Joly et al. prepared cross-linked cellulose plastic film by SM reaction[183]). However, the current research showed the adaptability of the CM reaction under emulsion conditions. As many polysaccharides and their derivatives are water-soluble but poorly soluble in good CM

solvents (e.g. THF, and dichloromethane), it is of great value to be able to perform the reaction in such heterogeneous and aqueous conditions. Also, as the Edgar group has proven that long chain terminally olefinic cellulose esters (e.g. cellulose pent-4-enoate and cellulose undec-10-enoate) are suitable for CM with a variety of CM partners, the results here showed the possibility of using acrylate as a handle on the polysaccharide backbone for CM reactions. If this is the case, the use of this type II/III olefin as a handle would avoid SM and thus unwanted crosslinking between polysaccharide acrylates due to the low reactivity of acrylate olefin towards SM. This could also make it possible to obtain high conversion to CM products using CM partners in equimolar amounts or in only slight molar excess. It is worthy to mention that although no such cases have been reported yet, polysaccharide modification by CM could also possibly be performed in aqueous solution homogenously with careful selection of water-soluble olefin metathesis catalysts[188,189] and corresponding CM partners.



a b

Figure 2.34. a) Schematic representation of the interfacial reaction between acrylated dextran **1** and phenyldi(undec-10-en-1-yl)-phosphate **2** leading to a cross-linked polymer network **3**. b) Schematic representation of the interfacial olefin cross-metathesis at the water-oil interface of a nanodroplet in an inverse miniemulsion process for the formation of stable nanocapsules. Adapted with permission from Ref. 187[187]. Copyright 2014, American Chemical Society.

Although researchers in the realms of both synthetic polymers and polysaccharides have shown the modular and efficient nature of the CM chemistry, it may not perfectly fit the definition of a polymer chemistry "click" reaction. According to a definition of click reactions

adapted to the context of polymer chemistry by Barner-Kowollik et al.[190] in 2011, the requirements for a "click" polymer chemistry include equimolarity, large-scale purification, and fast reaction rate, in addition to the requirements in the broader Sharpless' click definition such as those for chemoselectivity and stable products. Clearly, the CM reaction investigated by the Edgar group and other groups fulfills most of these requirements, as it is modular, rapid, chemoselective, and wide in scope. However, the CM products are sometimes unstable due to the reactive α,β-unsaturated double bond, and so require the addition of free radical scavenger or a further hydrogenation step. Neither does the CM reaction meet the requirement of equimolarity, as an excess amount of the CM partner is usually needed to drive to high conversion and selectivity of CM over SM. Therefore, we feel that describing this reaction as "click-like" rather than "click" would be appropriate and accurate.

2.8 Conclusions and prospects

Advances in organic chemistry have provided to polysaccharide chemists some useful synthetic tools for side chain modification, synthesis of polysaccharide-based graft or block copolymers, polysaccharide-based networks, as well as modification of polysaccharide surfaces. For example, the advent of carbonyl activating reagents such as DCC and CDI has enabled mild and efficient esterification of polysaccharides, while the development of protection-deprotection chemistry and other strategies have made possible regioselective modification of polysaccharides. Although these developments have expanded the potential of polysaccharide chemistry and materials, until recently the chemical functionality and architectural diversity of polysaccharide derivatives have still been largely restricted by limitations of conventional synthetic pathways such as esterification and etherification.

Click chemistry, the concept of which was coined less than 20 years ago by Sharpless and coworkers, provides unprecedentedly powerful tools for design and synthesis of novel polysaccharide derivatives in a more controlled and modular manner. Copper catalyzed azide/alkyne cycloaddition, also known as CuAAC, is one of the first of the click reactions that was introduced to polysaccharide chemistry and helped to generate a variety of families of novel derivatives. This reaction, characterized by efficient and orthogonal azide/alkyne coupling under mild reaction conditions using Cu^{I} catalysis, however, has its own limits, such as the toxicity of copper, the explosive and toxic potential of azides, and chain scission induced by reactive oxygen species (ROS), when used in areas including polysaccharide modification.

The development and implementation of other click reactions including metal-free [3+2] cycloaddition reactions, Diels-Alder reactions, thiol-Michael and thiol-ene reactions, and the oxime reaction have, to a large degree, helped polysaccharide chemists surpass the limitations associated with CuAAC, and allowed synthesis of some novel polysaccharide conjugates, copolymers, and networks. Besides the advantage inherent in metal-free coupling, these reactions also add valuable traits to polysaccharide chemistry. Strain-promoted alkyne/azide cycloaddition (SPAAC), azide/oxanorbornadiene cycloaddition and especially the most recently unveiled inverse electron demand Diels-Alder (iEDDA) not only serve as synthetic tools for modification, but also allow some sophisticated applications such as *in vivo* labeling, due to their metal-free nature and their fast reaction kinetics. Hetero Diels-Alder reactions employing thiocarbonylthio compounds smoothly combine polysaccharide chemistry, click chemistry and RAFT polymerization, and so provide an ideal pathway for the synthesis of polysaccharide-based graft or block copolymers. The recent application of olefin cross-metathesis to polysaccharide modification is also a "click-like" reaction, as it shares a number of features with click chemistry, such as rapid, efficient and modular coupling under mild conditions, easy purification, absence of offensive byproducts, and high yields, with only the requirement for high molar excess of the metathesis partner significantly differing from the definition by Barner-Kowollik et al. of a polymer click reaction[190] (the instability of the products to free radical reactions is another difference, but one that is easily correctable by hydrogenation, even in a one-pot reaction).

Application of modern click chemistry to the field of polysaccharide modification as described in this review has opened many new doors, enabling the science of modification of these diverse, abundant, and renewable materials to move to a new era of more controlled, modular, and chemoselective synthesis. These new additions to the toolbox of polysaccharide chemists make it certain that vastly increasing numbers and varieties of polysaccharide derivatives with unique and superior properties will be synthesized efficiently and will be exploited towards the goal of a more sustainable world.

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Chapter 3: Hydroboration-oxidation: A chemoselective route to cellulose ω-hydroxyalkanoate esters

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Abstract:

We describe the first synthesis of hydroxy-functionalized polysaccharide esters via chemoselective olefin hydroboration-oxidation in the presence of ester groups. Cellulose esters with terminally olefinic side chains were first synthesized by esterification of commercially available cellulose esters (e.g. cellulose acetate) with undec-10-enoyl chloride or pent-4-enoyl chloride. Subsequent two-step, one-pot hydroboration-oxidation reactions of the cellulose esters were performed, using 9-borabicyclo[3.3.1]nonane as hydroboration agent, followed by oxidizing the intermediate borane to a hydroxyl group using mildly alkaline H_2O_2. Sodium acetate was used as a weak base to catalyze the oxidation, thereby minimizing undesired ester hydrolysis. Characterization methods including FTIR, ^{1}H, and ^{13}C NMR proved the selectivity of the hydroboration-oxidation pathway, providing a family of novel cellulose ω-hydroxyalkanoyl esters that were previously difficult to access.

Keywords: 9-BBN; sodium acetate; cellulose ester; olefin; hydroboration; alcohol.

3.1 Introduction

Polysaccharides are abundant, diverse, and renewable biomaterials, which have an enormous range of structures and functions in nature. Selective chemical modification of these biopolymers is of great use to society, forming the basis of successful industries based on polysaccharide derivatives. These derivatives are typically more readily processed than the natural materials, and can be designed to possess properties fine-tuned for specific applications[1]. Among such derivatives, hydroxy-substituted polysaccharides, especially some of the hydroxy-substituted cellulose derivatives (e.g. hydroxypropyl methylcellulose, HPMC), possess important properties that have made them valuable materials in commerce[1]. The grafted hydroxyl groups enhance the hydrophilicity of the derivative, while the relatively randomly substituted cellulose

hydroxyalkyl ethers are much less crystalline than the parent cellulose. The reduced crystallinity and enhanced hydrophilicity render these hydroxyalkyl ethers water-dispersible or water-soluble, allowing easy processing and also opening up additional applications, e.g. in aqueous rheology modification[2]. Such properties have been exploited in fields like drug delivery, where ethers such as HPMC are used to modify release kinetics or amorphous/crystalline morphology of an incorporated drug[3], and in waterborne coatings where hydroxyethyl cellulose for example is used to impart shear-thinning and enhanced pigment dispersion to latex house paints[1]. Hydroxy-substituted polysaccharides have the ability to interact with molecules such as drugs and other polymers via hydrogen bonding. Alone or paired with other suitable polymers, such cellulose derivatives are promising excipients for oral drug delivery. For example, HPMC and hydroxypropyl methylcellulose acetate succinate (HPMCAS) are frequently used in amorphous solid dispersion (ASD) formulations with hydrophobic drugs to enhance drug solution concentrations[4,5]. Furthermore, as the pendent hydroxyls tend to have less hindered approach angles than those of the native polysaccharide that are directly appended to the ring carbons, it makes them good sites for further reactions[6].

Several methods have been developed for synthesis of hydroxy-substituted cellulose derivatives. Among these methods, ring-opening of epoxides (e.g. propylene oxide and ethylene oxide) by deprotonated cellulose hydroxyls (NaOH) is the most commonly used strategy to prepare hydroxyalkyl ethers of cellulose. Important cellulose derivatives synthesized in this way include 2-hydroxypropyl cellulose (HPC), 2-hydroxyethyl cellulose (HEC), and mixed cellulose ethers or ether-esters such as HPMC and HPMCAS[1,7]. However, the epoxide ring-opening methods are imprecise in terms of product structure in two respects. First, reaction with an epoxide gives a new alkoxide distal from the main cellulose chain, less hindered and more reactive, thereby causing oligo(hydroxyalkyl) side chains of various lengths to be formed. Secondly, while ethylene oxide is symmetrical, many epoxides (e.g. propylene oxide) have two different sites for potential nucleophilic attack, leading to a mix of positional isomers. Therefore synthesis of hydroxyl-containing cellulose derivatives by classical epoxide ring-opening methods leads to complex mixtures of products that are very difficult to fully characterize; consequently, product structure and uniformity cannot be fully controlled.

Cellulose esters with pendant hydroxyl groups are interesting synthetic targets. Due to the hydrolytic instability of ester bonds, these polymers will have alternative biodegradability

pathways[8] compared with their ether counterparts. However, for several reasons a simple synthetic pathway to cellulose ω-hydroxyalkanoates has not yet been created. As terminal alcohols are reactive towards acylation, direct esterification of cellulose using an activated acyl reagent that also possessed terminal alcohol functionality would more likely lead to formation of an oligo/polyester, either homopolymeric and/or grafted onto the cellulose backbone. While protecting group techniques may solve this issue, protection and deprotection can be expensive, laborious, and may be plagued by unwelcome reactivity of protected intermediates[9]. Cellulose ω-hydroxyalkanoates could in theory also be synthesized by ring-opening reactions between cellulose and lactones[10,11]. However, the literature shows that in fact polylactones (e.g. polycaprolactone) become grafted onto cellulose under such conditions; thus the steric freedom and resulting higher reactivity of the initially formed w-hydroxyl group are an issue in this approach as well. Generation of polyester homopolymers is a competing side reaction that also introduces complexity and inefficiency.

In previous studies[12-14], our group discovered that olefin cross-metathesis chemistry (CM) can be successfully applied to polysaccharides to synthesize discrete, non-crosslinked derivatives, enabling mild and modular side-chain modification of polysaccharides to afford products with carboxylic acid and a variety of other terminal functionalities. In the CM approach, terminally olefinic cellulose esters react with other functionalized alkenes under ruthenium catalysis[12], and thus incorporate the corresponding functional group onto the cellulose side-chain. However, the CM approach for synthesis of cellulose ω-hydroxyalkanoates may be inefficient since the required allyl alcohol CM substrates may be prone to self-metathesis, leading to low CM conversion[12].

Therefore investigation of potentially efficient methods for synthesis of discrete cellulose ω-hydroxyalkanoates is appealing. Hydroboration followed by oxidation is a well-known method in small molecule chemistry that can convert terminal olefins to primary alcohols by the net addition of H_2O across the double bond[15]. Hydroborating agents such as diborane add to alkenes to form organoboranes in an *anti*-Markovnikov manner[16]. Subsequent oxidation with hydrogen peroxide (usually under alkaline conditions) gives the corresponding anti-Markovnikov alcohols in which borone has been replaced by OH. Among such borane derivatives, 9-borabicyclo[3.3.1]nonane (9-BBN) exhibits remarkable thermal stability and also excellent regioselectivity, providing almost exclusively primary alcohols in hydroboration-oxidation of

simple terminal alkenes[16]. Standard hydroboration-oxidation conditions involve reacting a terminal alkene with an organoborane reagent in THF, and subsequent oxidation of the intermediate borane by the addition of H_2O_2 under alkaline conditions (e.g. NaOH). Heinze and coworkers[17] synthesized 3-allylcellulose by reaction of a regioselectively protected 3-hydroxycellulose with allyl halide, then converted the allyl group to a 3'-hydroxypropyl group by reaction with 9-borabicyclo[3.3.1]nonane (9-BBN), and subsequent alkaline oxidation. This approach teaches us how to efficiently synthesize w-hydroxyalkylcellulose ethers. However, we face additional challenges when considering a similar approach to synthesis of cellulose ω-hydroxyalkanoates. First, it is unknown whether 9-BBN would reduce cellulose esters. Hydroboration pioneer H.C. Brown has shown that small molecule esters are reduced, albeit slowly, to primary alcohols by diborane[18]. Ester reduction by 9-BBN was also studied by Brown, who showed that 9-BBN will also reduce esters slowly[19]; Brown has described selective reductions of ketones in the presence of esters by taking advantage of the faster reduction rate by 9-BBN of ketones vs. esters[20]. Secondly, an alkaline reagent (usually NaOH) is used in typical oxidation steps of hydroboration-oxidation reactions. Cellulose esters are quite labile to alkaline hydrolysis, with saponification usually occurring very rapidly under even the mildest conditions[21]. Therefore there were at least two good reasons to be skeptical that this approach to cellulose ω-hydroxyalkanoates would prove successful. Herein, we report efforts to employ hydroboration-oxidation of terminally olefinic cellulose esters in the synthesis of cellulose 5'-hydroxypentanoate and 11'-hydroxyundecanoate esters. We also describe our efforts to suppress ester hydrolysis during both the hydroboration and oxidation steps.

3.2 Experimental:

3.2.1 Materials

Cellulose acetate (CA-320S, M_n 38.0 kDa, DS(Ac) 1.82), cellulose acetate propionate (CAP-504-0.2, M_n 15.0 kDa, DS(Pr) 2.09, DS(Ac) 0.04), and cellulose acetate butyrate (CAB-553-0.4, M_n 20.0 kDa, DS(Bu) 1.99, DS(Ac) 0.14) were kindly donated from Eastman Chemical Company. The molecular weight information was reported by the supplier and the DS values were previously measured by our group[22]. Triethylamine (TEA) and 1,3-dimethyl-2-imidazolidinone (DMI) were purchased from Acros Organics. Sodium acetate (NaOAc), anhydrous tetrahydrofuran (THF), hydrogen peroxide solution (30% w/w in H_2O), 9-

borabicyclo-[3.3.1]-nonane (9-BBN, 0.5 M in THF), and methyl ethyl ketone (MEK) were purchased from Sigma-Aldrich. DMI was dried over 4 Å molecular sieves before use. All other purchased reagents were used as received.

3.2.2 Preparation of terminally olefinic cellulose esters

The synthesis of terminally olefinic cellulose esters was according to our previous publications[12,13] with slight modification.

(1) Example procedure of the synthesis of *cellulose acetate undec-10-enoate* (**1**, **CA-Un067**)

Cellulose acetate (CA-320S, 1.00 g, 4.19 mmol AGU) was dissolved in DMI (30 mL), and the solution was heated to 90 °C with mechanical stirring under N_2. Triethylamine (1.29 mL, 9.22 mmol, 2.2 eq/AGU) was added. A condenser was used to avoid evaporative loss of the base catalyst. Undec-10-enoyl chloride (1.70 g, 8.36 mmol, 2.0 eq/AGU) was added dropwise and allowed to react at 90 °C for 20 h. The reaction mixture was then cooled to room temperature, filtered, and the filtrate was precipitated in 300 mL of a 50:50 v/v water–ethyl alcohol solution. The precipitate was redissolved in a minimal amount of CH_2Cl_2 (~ 5 mL) and reprecipitated in hexane (~ 100 mL). The product was washed with hexane and dried under vacuum at 40 °C (yield: 1.30 g, 95%, white powder).

A similar procedure was adopted in the synthesis of cellulose acetate pent-4-enoate (**2**, **CA-Pen056**) using 2 eq of pent-4-enoyl chloride per 1 eq AGU (yield: 1.12 g, 94%, light yellow powder).

(2) Example procedure for the synthesis of *cellulose acetate propionate undec-10-enoate* (**3**, **CAP-Un057**).

Cellulose acetate propionate (CAP-504-0.2, 1.00 g, 1.78 mmol AGU) was dissolved in MEK (20 mL), and the solution was heated to 60 °C with magnetic stirring under N_2. After the addition of triethylamine (1.08 mL, 3.92 mmol, 2.2 eq/AGU), undec-10-enoyl chloride (1.44 g, 7.12 mmol, 2.0 eq/AGU) was added dropwise, and the mixture was stirred for 20 h at 60 °C. After cooling to room temperature, followed by filtration to remove triethylammonium chloride, the filtrate was precipitated into 300 mL of a 50:50 v/v water/ethyl alcohol solution. The product was redissolved in 20 mL of CH_2Cl_2, reprecipitated in 200 mL of hexane, and dried under vacuum at 40 °C (yield: 1.16 g, 86%, white powder).

A similar procedure was followed for the preparation of cellulose acetate butyrate undec-10-enoate (**4**, **CAB-Un059**) using 2 eq of undec-10-enoyl chloride per 1 eq AGU (yield 1.03 g, 71%, white powder).

3.2.3 Hydroboration-oxidation procedure for the preparation of hydroxy-substituted cellulose esters.

1, **CA-Un067** (0.50 g, 0.97 mmol double bond) was dissolved in a minimum amount of anhydrous THF (~5 mL) in a water-jacketed flask at 0 °C under N_2. Under stirring, 19.4 mL of 0.5 M 9-BBN in THF (9.7 mmol) was added dropwise into the mixture (after addition, gelation may occur). After 12 h, 3.4 mL of 3 M NaOAc in water (10.2 mmol) was added, followed by the addition of 20 mL of H_2O_2 solution. The reaction mixture was then stirred for 24 h at 25 °C, dialyzed against water for 2 d, filtered and dried under vacuum to give product **6** (yield: 0.44 g, 87%, white powder).

Similar procedures were adopted in the hydroboration-oxidation other reactions to give the corresponding products listed in **Table 3.2**.

3.2.4 Measurements.

^{1}H NMR spectra were acquired on a Bruker Avance 500 spectrometer operating at 500 MHz with 16 scans. Samples were analyzed as solutions in $CDCl_3$ or DMSO-d_6 (ca. 10 mg/mL) at 25 °C in standard 5 mm o.d. tubes. Three drops of trifluoroacetic acid were added to shift the water peak downfield from the spectral region of interest. ^{13}C NMR spectra were obtained on a Bruker Avance 500 spectrometer with a minimum of 5000 scans in DMSO-d_6 (ca. 50 mg/mL) at 25 °C. To obtain the T_g values of the cellulosic polymers, DSC was performed on a TA Instruments Q100 apparatus with a heat/cool/heat cycle. Dry powders (ca. 5 mg) were loaded in TA hermetic aluminum pans. Each sample was equilibrated at −50 °C before ramping to 160 °C at the rate of 20 °C/min, followed by quenching to −50 °C and ramping to around 200 °C at a rate of 20 °C/min; glass transition data was acquired during the second heating scan. FTIR spectra were obtained on a Nicolet 8700 instrument. Size exclusion chromatography (SEC), if not otherwise specified, was performed on Agilent 1260 Infinity Multi-Detector SEC using DMAc with 0.1 M LiCl as the mobile phase (50 ºC) with 3 PLgel 10 μm mixed-B 300 × 7.5 mm columns in series. A system of multiple detectors connected in series was used for the analysis. A multi-angle laser light scattering (MALS) detector (DAWN-HELEOS II, Wyatt Technology

Corporation, Goleta, CA), operating at a wavelength of 658 nm, a viscometer detector (Viscostar, Wyatt Technology Corporation, Goleta, CA), and a refractive index detector operating at a wavelength of 658 nm (Optilab T-rEX, Wyatt Technology Corporation, Goleta, CA) provided online results. Data acquisition and analysis was conducted using Astra 6 software (Wyatt Technology Corporation, Goleta, CA). Monodisperse polystyrene standard (Mw ~ 21k, Đ ~ 1.02) was run first in every sample series for the purpose of calibration and confirmation.

3.3 Results and Discussions:

The hydroboration-oxidation approach has been proven successful by the Heinze[17] and Kadla groups[6] for the synthesis of w-hydroxyalkylcellulose ethers. However, the potential difficulties cited above for applying this method to the synthesis of cellulose ω-hydroxyalkanoates have perhaps discouraged progress in this area. To explore whether these difficulties could be circumvented, we first synthesized cellulose esters with terminally unsaturated side-chains through esterification between commercial cellulose esters and acyl chlorides (**Table 3.1**) to obtain cellulose pent-4-enoate or undec-10-enoate esters. Terminally unsaturated side-chains with two chain lengths (5 or 11 carbons) were added to three different commercial cellulose esters, and the terminal olefins would serve later as handles for subsequent hydroboration.

Table 3.1. Synthesis of cellulose esters with terminally unsaturated side-chains.

Sample #	**Abbr.**[a]	**Solvent**	**Temp (ºC)**	**Mol. ratio**[d]	**DS**[e]	**DS(other)**	**M_n(kDa)/DP**	*Đ*	**T_g (ºC)**
1	CA-Un067	DMI[b]	90	2.0	0.67	Ac 1.85	49.8/144	1.69	127
2	CA-Pen056	DMI	90	2.0	0.56	Ac 1.82	37.4/131	1.40	162
3	CAP-Un057	MEK[c]	60	2.0	0.57	Ac 0.04 Pr 2.09	13.9/37	2.44	70
4	CAB-Un059	MEK	60	2.0	0.59	Ac 0.14 Bu 1.99	37.2/92	2.32	70

a. *CA*, *CAP* and *CAB* in the abbreviations stand for the cellulose esters CA-320S, CAP-504-0.2 and CAB-553-0.4; *Un* or *Pen* indicates the terminally olefinic side-chain on the cellulose

derivative comes from acylation with undec-10-enoyl chloride or pent-4-enoyl chloride respectively; the number (e.g. 067 in CA-Un067) indicates the DS of the terminally olefinic side chain (DS=0.67 for sample CA-Un067). b. DMI: 1, 3-dimethyl-2-imidazolidinone. c. MEK: methyl ethyl ketone. d. Mol of acylation reagent (undec-10-enoyl chloride or pent-4-enoyl chloride) per mol of anhydroglucose unit. e. Degree of substitution of the terminally olefinic side chain.

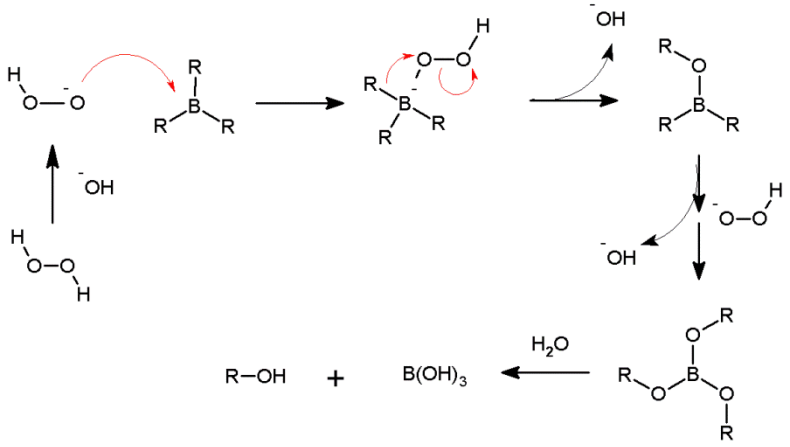


Figure 3.1. Mechanism of oxidation of organoboranes using alkaline hydrogen peroxide[23]

The hydroboration of interest is the addition of a borane (R_2B-H) species, 9-BBN in this case, across a terminal C=C double bond regioselectively, in anti-Markovnikov[24] fashion, forming a carbon-boron bond which will be subsequently converted to a carbon-oxygen bond by oxidation. Alkaline hydrogen peroxide has been widely used for oxidation of organoboranes[25,26]. In the presence of alkali, hydroperoxide anion is generated as the active oxidant. With hydroperoxide anion adding to the empty orbital of the borane, intramolecular rearrangement occurs to give borate esters, which may undergo hydrolysis to afford boric acid and the corresponding terminal alcohol (**Figure 3.1**)[23].

We first conducted hydroboration-oxidation of cellulose acetate undec-10-enoate (**1**, **Figure 3.2**, and **Table 3.2**) with 9-BBN following the procedure reported by the Heinze group[17], in which NaOH was employed as an alkaline catalyst during the oxidation step. However, unsurprisingly the product (**5**) was not soluble in common organic solvents including THF, chloroform, DMSO and DMF. Under such reaction conditions, alkaline hydrolysis of the cellulose ester side chains is predictable. Peracetylation of the product, during which most of the free hydroxyl groups were esterified to acetate[27], improved its solubility in organic solvents. The

^{1}H NMR spectrum of the peracetylated product (Supporting Information **S3.7**) showed close similarity with that of cellulose triacetate, indicating that under these strongly alkaline oxidation conditions, most, if not all, of the ester side-chains were cleaved, leading to cellulose as the final product. Although many functional groups including some esters[25,28-30] have been shown to survive strongly alkaline conditions in oxidation of small molecule boranes, this is apparently not the case with polysaccharide esters, at least under the conditions we investigated.

We examined the possibility of using the reactive and simple hydroboration reagent diborane in THF, at the same time employing sodium acetate (3 M in water) as a potential alkaline catalyst for oxidation in order to avoid unwanted ester reduction. In previous studies with small molecules[31], NaOAc had been employed in hydroboration-oxidation reactions as a weaker base, in that case successfully preventing hydrolysis of ester linkages. However, the proton NMR spectrum of product **7** showed obvious loss of ester substituents on the cellulose backbone (Supporting Information **S3.10**). We suspected that this might indicate that the reactive diborane may be capable of reducing the ester groups resulting in ester cleavage, rather than hydrolysis during the oxidation step, catalyzed by the weak base NaOAc . Such a result would not be unexpected, as studies in the Brown group have showed the ability of diborane to reduce ester carbonyls[32-34].

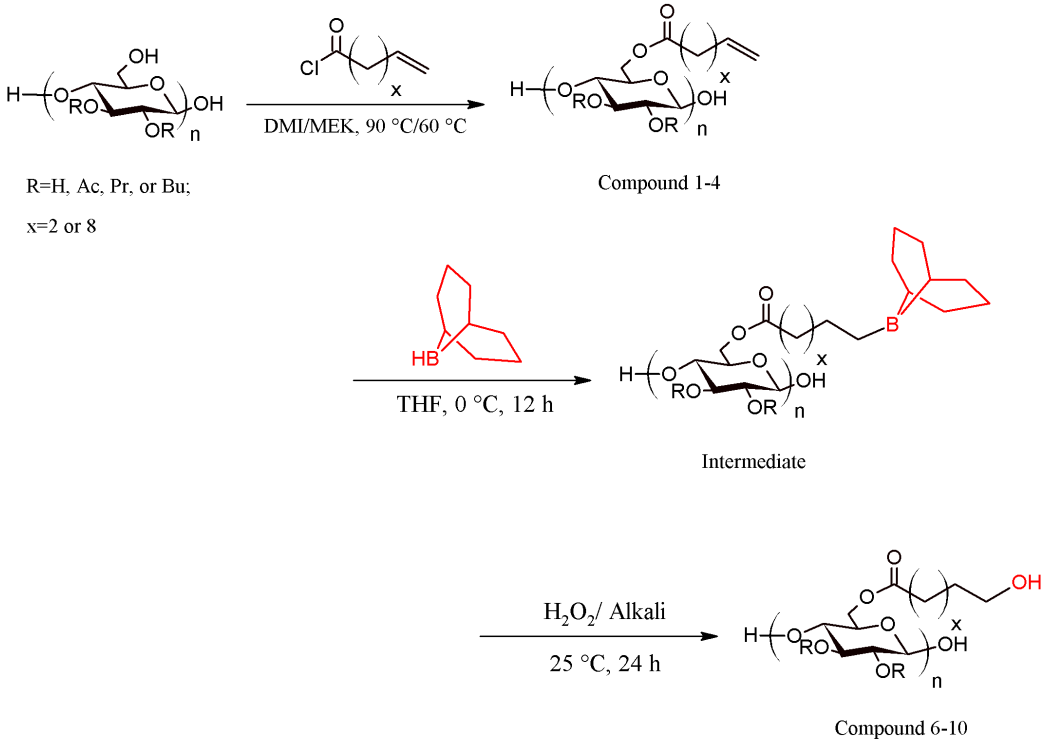


Figure 3.2. General scheme for the synthesis of hydroxy-substituted cellulose esters by hydroboration-oxidation reaction.

The results described above of reactions using 9-BBN/NaOH and diborane/NaOAc combinations confirmed our previous concerns that the hydroboration reagent and the base catalyst may not preserve the ester linkages of cellulose esters in hydroboration-oxidation reactions. Compared with diborane, 9-BBN is more sterically hindered, and therefore would be expected to reduce ester groups at much lower rates. Previous studies have shown that 9-BBN possesses higher selectivity towards reduction of different functional groups (e.g. rapid hydroboration of olefins, slow ester reduction)[19]. To circumvent the undesired ester loss caused either by ester saponification or borane reagent induced ester reduction, we hypothesized that conditions employing 9-BBN as the hydroboration reagent and NaOAc as the oxidation catalyst might lead to desired products. This combination was employed in subsequent experiments as shown in **Table 3.2**. We performed the reaction on various terminally olefinic cellulose esters to verify its broad applicability, but cellulose acetate undec-10-enoate (**1**, CA-Un067) will serve to exemplify the results obtained. From FTIR spectra (**Figure 3.3**), the peaks at 1643 and 3073 cm^{-1} in the spectrum of CA-Un067, assigned to C=C stretch and =C-H stretch of the terminal olefin, are absent in the spectrum of the product **6**, Hb-CA-Un067, indicating the total transformation of the terminal alkene. On the other hand, the peak at 1751 cm^{-1}, assigned to the ester C=O stretch, still exists after the reaction, indicating the successful preservation of the ester linkage.

We further pushed the concept of reduced alkalinity during the oxidation step by employing an equal volume of water rather than NaOAc solution. Surprisingly aqueous H_2O_2 alone, without any added alkali, was also successful, affording a product (**6'**) with almost identical IR, NMR and SEC profile to those of Hb-CA-Un067. Given the accepted oxidation mechanism, it is intriguing that the reaction was successful in the complete absence of base. Measurements of pH (**S3.14**) showed that all the different reaction media with NaOAc, H_2O or NaOH were acidic with pH values 5.47, 3.12 and 6.76 respectively, which may be attributed to the acidic nature of H_2O_2. According to our results, all of the three "bases" successfully catalyzed the oxidation reaction of the organoborane intermediate (although side reactions ester hydrolysis and/or reduction occur in some cases as mentioned above). It is likely that a catalytic concentration of hydroperoxide anion is sufficient to enable the oxidation of organoboranes, as long as the reaction is allowed for a long enough period of time (24 h in our experiments). Since

the NaOAc conditions were simple, mild, and successful, and since we felt that the presence of NaOAc buffer could be beneficial in some cases, we used NaOAc for the rest of the reactions studied.

Table 3.2. Synthesis of hydroxy-substituted cellulose esters via hydroboration-oxidation

Product #	**Abbr.**[a]	**Starting cellulose ester**	**Alkaline catalyst**	**Hydroboration reagent**	**M_n (kDa)/DP**	*Đ*	**T_g**
5	NA	1/CA-Un067	NaOH	9-BBN	NA	NA	NA
6	Hb-CA-Un067	1/CA-Un067	NaOAc	9-BBN	40.9/138	1.90	118
6'	NA	1/CA-Un067	H_2O	9-BBN	38.0/128	1.67	113
7	NA	1/CA-Un067	NaOAc	Diborane	NA	NA	NA
8	Hb-CA-Pen056	2/CA-Pen056	NaOAc	9-BBN	37.8/128	1.89	138
9	Hb-CAP-Un057	3/CAP-Un057	NaOAc	9-BBN	13.6/35	2.75	40
10	Hb-CAB-Un059	4/CAB-Un059	NaOAc	9-BBN	50.7/122	3.37	63

a. Abbreviations using the same rule as showed in **Table 3.1**; *Hb* indicates product after hydroboration-oxidation.

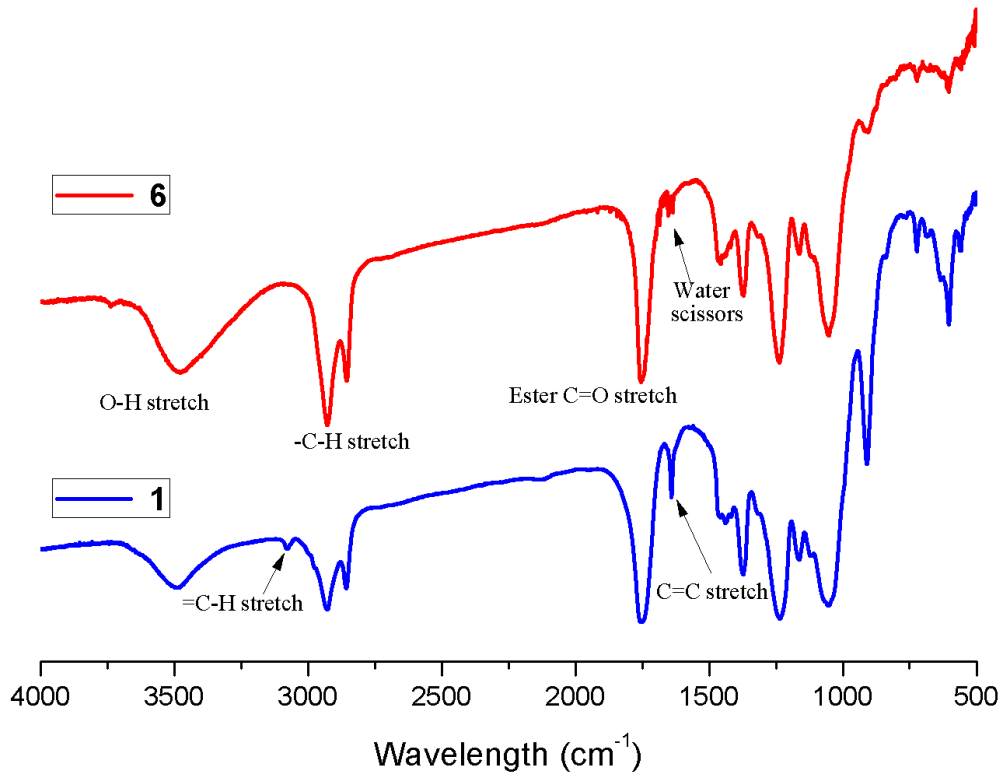


Figure 3.3. FTIR spectra of cellulose acetate undec-10-enoate (**1**, CA-Un067) and the corresponding hydroboration-oxidation product (**6**, Hb-CA-Un067).

^{1}H NMR proved to be a useful technique both for monitoring reaction progress and to calculate DS of the product side-chain substituents. The proton NMR spectrum of Hb-CA-Un067 (**Figure 3.4**) shows disappearance of the terminal olefin signals of CA-Un067 at 4.90 and 5.75 ppm after the reaction, indicating complete conversion of the terminal double bond. Correspondingly, conversion of terminal olefin to primary w-hydroxyalkyl was supported by the emergence of a new multiplet at 3.37 ppm in the spectrum of Hb-CA-Un067, which was assigned to the C-13 methylene protons that are geminal to the w-hydroxyl group. The ^{13}C NMR spectrum (**Figure 3.5**) supports this conclusion. Terminal olefin carbon signals of CA-Un067 at 114 and 139 ppm disappeared completely after the reaction, while a new peak assigned to the hydroxyl-bearing w-methylene carbon appeared at 61 ppm, supporting high conversion of the terminal olefin to primary alcohol. It is noteworthy that there were still small signals in both

proton and carbon product NMR spectra that could be attributed to residual 9-BBN moieties, as also illustrated in the ^{1}H-^{13}C HSQC spectrum of Hb-CA-Un067 (**S3.11**). Considering that the oxidation step was done under heterogeneous conditions (upon the addition of H_2O_2 and base catalyst, the polymer at least partially precipitates), it was likely that a small amount of the borane adduct was not well accessible by H_2O_2 and OH^- due to phase issues, and thus was less available for oxidation.

It was gratifying that both FTIR and NMR spectra of CA-Un067 (**1**) and Hb-CA-Un067 (**6**) indicated successful hydroboration/oxidation of terminal olefin to primary alcohol, and gave preliminary indication of preservation of the ester linkages. We wished to determine the exact DS of both the acetate and ω-hydroxyalkanoate ester moieties, as well as the percent conversion of terminal olefin to primary alcohol, but it was a challenge to use typical ^{1}H NMR methods to calculate these DS values due to ^{1}H NMR signal overlap. To solve this problem, we peracetylated **6** (**Figure 3.4**, 6-peracetylated). Upon peracetylation, all the free hydroxyl groups (both residual glucose ring OH groups and the w-OH groups from hydroboration) can be esterified to corresponding acetate[27], thus making the DS of hydroxyalkanoate (($DS_{(Hydro)}$)) and the DS of alkanoate with 9-BBN moiety ($DS_{(Residual)}$) the only variables. Based on the integrals of signals in spectra of **6** and **6**-peracetylated in **Figure 3.4**, and the equations:

(1) From spectrum of **6** and **6**-peracetylated: $\frac{0.02}{1.00} = \frac{2\times DS(Residual)}{7+2\times DS(Hydro)}$

(2) From spectrum of **6**: $\frac{2.21+0.02}{1.00} = \frac{3\times DS(Ac)+18\times DS(Hydro)+14\times DS(Residual)}{7+2\times DS(Hydro)}$

(3) From spectrum of **6**-peracetylated: $\frac{2.60+0.02}{1.00} = \frac{(3-DS(Residual))\times 3+18\times DS(\square ydro)+14\times DS(Residual)}{7+2\times DS(Hydro)}$

$DS_{(Hydro)}$ was calculated as 0.66, while $DS_{(Residual)}$ was 0.08, and $DS_{(Ac)}$ was 1.85. Compared with the starting material (CA-Un067), no significant change in DS of ester side-chains was observed in its hydroboration-oxidation product Hb-CA-Un067, demonstrating the mild nature of this reaction. From the calculated DS values, about 90% of terminal olefin was converted to hydroxyl for product Hb-CA-Un067.

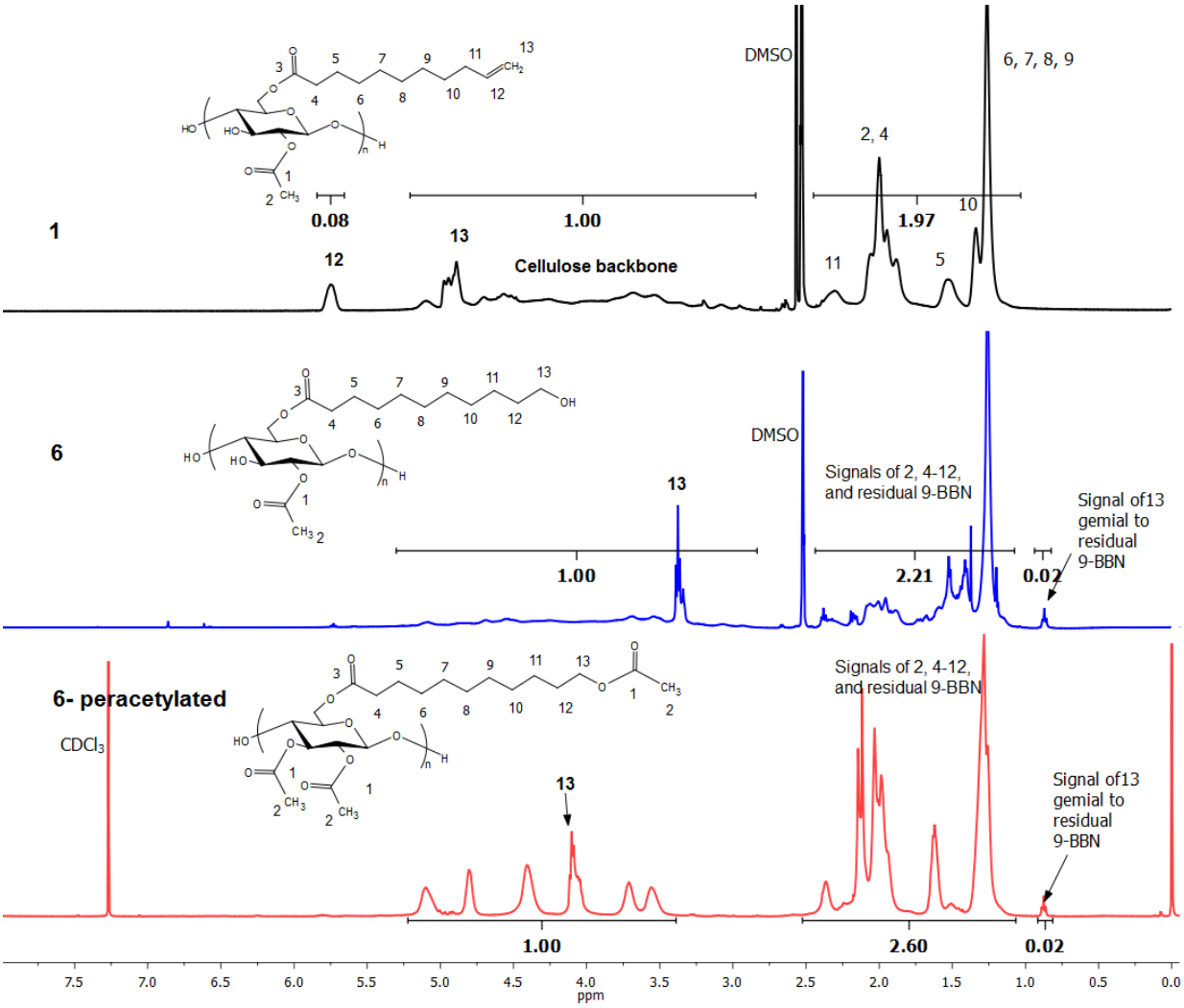


Figure 3.4. ^{1}H NMR spectra of CA-Un067 (**1**), the corresponding hydroboration-oxidation product Hb-CA-Un067 (**6**), and the subsequent peracetylation product (**6**-peracetylated).

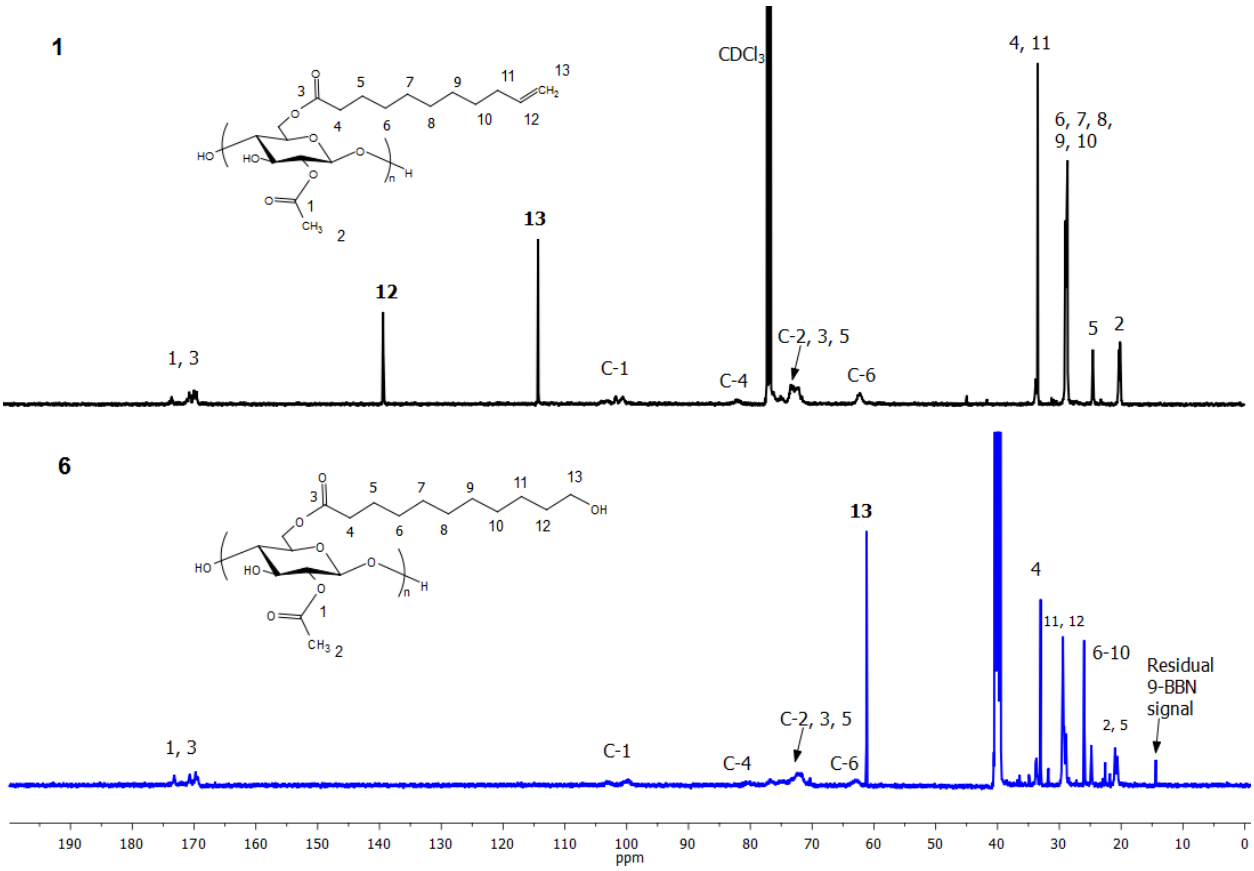


Figure 3.5. ^{13}C NMR spectra of CA-Un067 (**1**), and the corresponding hydroboration-oxidation product Hb-CA-Un067 (**6**).

Using similar reaction conditions, cellulose undec-10-enoates with other ester groups (CAP-Un057 (**3**), and CAB-Un059 (**4**)) were also successfully subjected to hydroboration-oxidation. As there are more than two types of ester substituent in each of these derivatives, it was difficult to accurately calculate the DS of each substituent. However, proton NMR and IR spectra (**S3.2, 3.3, 3.5** and **3.6**) support the disappearance of terminal olefins and the appearance of hydroxyl groups. Moreover, the ratios of integrals in the proton NMR spectra fit well with the theoretical values when assuming that no ester substituent loss occurs (**S3.8** and **S3.9**). These results, combined with the results of full structural analysis of the hydroboration product of cellulose acetate undec-10-enoate (*vide supra*), support the hypothesis that the mildness of the reaction preserves the integrity of other, more sterically hindered ester groups like propionate and butyrate.

With the success of this reaction for cellulose undec-10-enoates (**1, 3** and **4**), we wished to examine whether cellulose acetate pent-4-enoate (**2**, CA-Pen056) would also be an appropriate

substrate, given that it possesses a shorter olefinic side-chain (5 carbons) compared to **1** (11 carbons), potentially raising steric issues as a result of greater proximity to the bulky cellulose main chain. Both IR and NMR results (**S3.1** and **S3.4**) showed success in transforming the C-5 terminal olefin to primary alcohol by hydroboration-oxidation under the 9-BBN/($NaOAc/H_2O_2$) conditions described above.

SEC was used to determine changes in molecular weight that may have occurred during the hydroboration/oxidation reaction sequence. Comparing the molecular weights of the starting cellulose esters and those of the hydroboration-oxidation products (**Table 3.1** and **3.2**), only minimal change in DP was observed for all products, demonstrating that the hydroboration-oxidation sequence is also mild with regard to reactions at the anomeric linkages and resulting molecular weight reduction. The heterogeneous conditions in the oxidation step may also help reduce the opportunities for oxidative chain scission[35].

Glass transition temperature (T_g) is an important thermal property that reflects the molecular mobility and processability of the polymer. For example, in oral drug delivery applications polymeric matrices are used in amorphous solid dispersion (ASD) of otherwise poorly soluble drugs, in which a high T_g polymer (at least 50 ºC above ambient temperature) is preferred in order to restrict the mobility of drug molecules and thus keep them in the amorphous state, so as to enhance drug dissolution. We used DSC to measure the glass transitions of the cellulose ω-hydroxyalkanoates (**Table 3.1**, **3.2** and **S3.12**, **S3.13**). Upon converting the terminal olefin to primary hydroxyl group by hydroboration-oxidation, a slight decrease in T_g was observed for each sample. Meanwhile, longer side-chain derivatives (**1** and **6**) exhibited lower T_g compared with their shorter side-chain counterparts (**2** and **8** respectively), consistent with observations in related polymer systems[12]. Apart from Hb-CAP-Un057 (**9**) which has a T_g of 40 ºC, the new cellulose esters have adequately high T_g values for consideration as potentially useful ASD polymers.

3.4 Conclusions:

Cellulose derivatives containing ester groups that bear terminal primary alcohols (cellulose ω-hydroxyalkanoates) were synthesized from terminally unsaturated cellulose esters for the first time using a hydroboration-oxidation sequence. This simple approach involves esterification of cellulosic polymers with terminally unsaturated acyl groups, followed by

hydroboration with the selective hydroboration reagent 9-BBN in THF, and subsequent oxidation with neutral or mildly alkaline hydrogen peroxide. Substitution of sodium acetate for sodium hydroxide as alkaline catalyst in the hydrogen peroxide oxidation step provided mild reaction conditions, permitting successful oxidation with essentially complete protection of ester linkages against hydrolysis, while the use of hindered 9-BBN was sufficient to prevent any detectable ester reduction. Compared with cellulose derivatives possessing pendent hydroxyl groups obtained through previous synthetic pathways, these hydroxy-substituted cellulose esters prepared by the hydroboration-oxidation approach have defined structures (monomeric hydroxyl-containing substituents, rather than cellulose-*g*-oligo/polyols), and this is achieved without requiring any laborious protection-deprotection techniques. There is every reason to expect that this approach will be applicable to other polysaccharides as well. This chemistry will permit synthesis of polysaccharide w-hydroxy derivatives that are complimentary to w-carboxylic acid, w-carboxylate ester, and other functional derivatives that are available by olefin cross-metathesis and other approaches[12,13]. This is particularly useful since the starting material for olefin cross-metathesis and for olefin hydroboration-oxidation can be identical, providing direct comparisons of polysaccharide structures nearly identical in all aspects other than terminal functional group, in structure-property relationship studies. These new hydroboration-oxidation methods will provide families of hydroxy-substituted polysaccharide esters that will be of great interest for structure-property studies in amorphous solid dispersion for drug bioavailability enhancement[36,37], and in other demanding applications.

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Chapter 4: Olefin cross-metathesis as a source of polysaccharide derivatives: Cellulose ω-carboxyalkanoates

(Adapted from Meng, X.; Matson J. B. and Edgar, K. J. (2014), Olefin Cross-Metathesis as a Source of Polysaccharide Derivatives: Cellulose ω-Carboxyalkanoates. Biomacromolecules 2015 (1), 177-187)

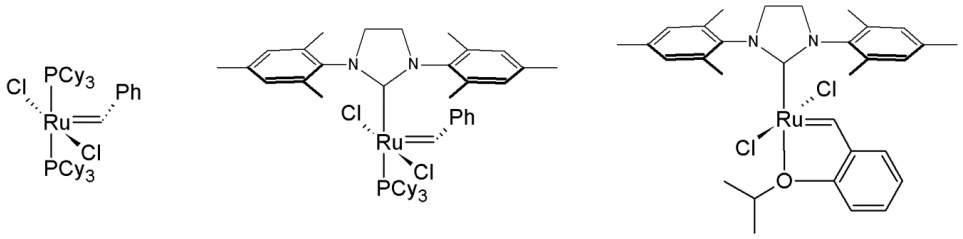
Abstract

Cross-metathesis has been shown for the first time to be a useful method for the synthesis of polysaccharide derivatives, focusing herein on preparation of cellulose ω-carboxyalkanoates. Commercially available cellulose esters were first acylated with 10-undecenoyl chloride, providing esters with olefin-terminated side chains. Subsequent cross-metathesis of these terminal olefin moieties with acrylic acid was performed in solvents including acrylic acid, THF and CH_2Cl_2. Complete conversion to discrete, soluble cross-metathesis products was achieved by using the Hoveyda-Grubbs' 2nd generation ruthenium catalyst and an excess of acrylic acid. Oligomerization during storage, caused by a free radical mechanism, was observed and successfully suppressed by the addition of a free radical scavenger (BHT). Furthermore, the cross-metathesis products exhibited glass transition temperatures (T_gs) that were at least 50°C higher than ambient temperature, supporting the potential for application of these polymers as amorphous solid dispersion matrices for enhancing drug aqueous solubility.

4.1 Introduction

Olefin metathesis has been developed in recent years as a powerful, versatile tool for the synthesis of complex small molecules, as well as the polymerization of olefinic monomers to create novel and useful polymeric structures.[1] In olefin metathesis, metal carbene complexes are used to rearrange double bonds in carbon skeletons with high functional group tolerance and under mild reaction conditions. Although ring closing metathesis (RCM) and ring opening metathesis polymerization (ROMP) have been comprehensively investigated over the past decade, olefin cross-metathesis (CM) has become an increasingly powerful tool in both organic and polymer chemistry thanks to the publication of Grubbs' model of selectivity for CM[2], and the development of active and selective CM catalysts.[3-5] It might have been expected that

polysaccharide chemists would quickly exploit these valuable new tools for synthesis of a wide variety of new polysaccharide derivatives, but this has not been the case. Only a few related studies have appeared; one example is the work by Reddy and co-workers,[6] who successfully cross-metathesized glucose-linked olefins with amino acid-appended olefins to construct the complete carbon skeleton of ieodoglucomides A and B, two unique glycopeptides isolated from marine-derived bacteria. What about similar CM reactions between, for example, a cellulose derivative bearing unsaturated side chains, and other olefin species, which could lead to a rich variety of otherwise inaccessible derivatives? Surprisingly, only a handful of studies[7-9] have described metathesis reactions of polysaccharide derivatives, and none of them have described successful CM. In a typical example, Joly et al.[8] observed self-metathesis (SM) of cellulose 10-undecenoate using Grubbs' catalyst (1st generation), driven by loss of the volatile ethylene co-product, affording crosslinked and insoluble cellulose plastic films. All such studies to date have reported dominant self-metathesis of olefin-substituted polysaccharides to afford crosslinked, insoluble products. Perhaps it is the negative results of these studies that have discouraged further investigation of cross-metathesis in polysaccharide derivatives.



C1 C2 C3

Figure 4.1. Commonly used Grubbs' catalysts for olefin metathesis.

In order to obtain discrete, soluble, polysaccharide-olefin CM products, SM between pendant terminal olefins must be absent, and there must be a high degree of conversion to CM products. Previous studies in other systems have shown that the type of catalyst (**Figure 4.1**) used influences the ability to obtain a high degree of conversion to cross-metathesis products. The Grubbs' 1st generation catalyst (**C1**) has proved to be insufficiently effective as a cross-metathesis catalyst in all but simple systems.[10,11] More reactive and thermally stable Grubbs' 2nd generation (**C2**) and Hoveyda-Grubbs' 2nd generation (**C3**) catalysts have been heavily studied for CM reactions. Compared with **C2**, the Hoveyda-Grubbs catalyst **C3** is more reactive towards

electron-deficient olefins, and it can initiate metathesis at lower temperature.[12] While the degree of conversion depends on the catalyst used, selectivity in CM is dependent primarily on the structure of the reacting olefin. Based on chemical structure and reactivity results, Grubbs empirically classified olefins into 4 types (**Table 4.1**).[2] Sterically-hindered and electron-deficient olefins of type II and III have low metathesis reactivity and only slowly homodimerize, while more reactive terminal olefins (type I) readily undergo homodimerization via metathesis. Moreover, the homodimers of the terminal olefins are susceptible to subsequent secondary CM reactions. As a result, when a type I olefin is reacted with a type II or III olefin, high conversion to a CM product can be achieved by employing an excess of the type II or III olefin.[13]

Table 4.1. Grubbs' categorization of olefins and rules for selectivity.[2]

Olefin Type	Olefin Metathesis Reactivity	Examples[a]
Type I	Rapid homodimerization, homodimer consumable	Terminal olefins
Type II	Slow homodimerization, homodimer sparingly consumable	Acrylates, acrylic acids, acrylamides
Type III	No homodimerization	3º Allylic alcohol (protected)
Type IV	Olefins inert to CM, but do not deactivate the catalyst (Spectator)	Vinyl nitro olefins

[a]Selectivity depends on catalyst used. The examples shown are valid for Grubbs' 2nd generation catalyst.

Grubbs' Rules:

Reactions between two olefins of Type I = Statistical CM

Reactions between two olefins of same type (non-Type I) = Non-selective CM

Reactions between two different types (except Type IV) = Selective CM

We were intrigued by the possible application of CM to the synthesis of cellulose derivatives containing carboxyl groups. The synthesis of such derivatives has been a long-standing and fascinating challenge. One issue is the problem of synthesizing derivatives with pendant carboxyl groups attached to a polysaccharide which also contains pendant hydroxyl groups; carrying out such a transformation under the acidic conditions commonly used to

manufacture cellulose esters is almost inevitably accompanied by crosslinking due to ester formation between chains. This restricts such nucleophilic substitution chemistry to near-neutral or alkaline conditions, which work well for simple cases like reaction of cellulose with succinic[14] or adipic anhydride[15]. Attachment of ω-carboxyalkanoates in which the intervening polymethylene chain is too long for facile cyclic anhydride formation is more complicated; in the past, it has been necessary to resort to protection/deprotection methodologies[16]. Such difficulties are unfortunate since polysaccharide ω-carboxyalkanoates have many useful properties that enable important applications. In coating applications, the carboxylic acid functionality renders the derivatives water-soluble or water-dispersible, enabling waterborne and high solids coatings systems, thereby reducing the use of volatile organic solvents and enhancing coatings performance.[17] Cellulose derivatives containing carboxyl groups are also important components of drug delivery systems.[18] Since carboxylic acids have pKa values in the range of 4-5, carboxyl-containing polysaccharides are protonated in the strongly acidic environment of the stomach, and are ionized in the near-neutral small intestinal milieu. This pH-sensitivity makes the derivatives good candidates for enteric polymeric coatings or matrices, which minimize drug/stomach exposure by preventing release until the formulation reaches the higher pH environment of the small intestine. Cellulose acetate phthalate (CAPhth)[19] was one of the first polymers used for such pH-sensitive, controlled release coatings in drug delivery. Other esters of cellulose with pendant carboxylic acid groups including cellulose acetate succinate (CAS)[20], hydroxypropyl methylcellulose phthalate (HPMCP)[21] and hydroxypropyl methylcellulose acetate succinate (HPMCAS)[22] have also proven interesting for enteric coating and controlled release.

Recent studies have shown the advantages of esters of cellulose with pendant carboxylic acids in delivery of poorly soluble compounds (Biopharmaceutical Classification System (BCS) Class II), by forming miscible blends of polymers and drugs, termed amorphous solid dispersions (ASDs). These molecularly dispersed drugs generate higher solution concentrations than achievable from the corresponding crystalline drugs, by maximizing drug surface area and eliminating the need for the drug to overcome its heat of fusion in order to dissolve. Supersaturated drug solutions generated from these ASDs can not only enhance the absorption of the drug from the gastrointestinal (GI) tract, but also provide a pH-controlled release profile. For example, HPMCAS has been proven to be an effective polymer for initiating and maintaining drug supersaturation in the GI tract, stabilizing the amorphous drug against crystallization,

thereby in some cases enhancing drug bioavailability.[23-25] More recently, the Edgar and Taylor groups jointly investigated the formation of ASDs of poorly water-soluble drugs and long-chain cellulose ω-carboxyalkanoates, i.e. cellulose adipate, suberate and sebacate derivatives, and found that some of these polymers were highly effective at generating and maintaining supersaturated drug solutions by inhibiting nucleation and subsequent crystal growth.[26-29] These studies also revealed that the DS of carboxylic acid functionality and the polymer hydrophobicity were key factors influencing the performance of the ASDs. The long side chains enhance the interactions of the polymers with hydrophobic drugs, while the pendant carboxylic acids provide both specific polymer-drug interactions and the pH-trigger for drug release through swelling of the ionized polymer matrix.

Herein, we report the synthesis of cellulose ω-carboxyesters through cross-metathesis of cellulose 10-undecenoate derivatives with acrylic acid. We describe optimization of reaction conditions and explore the impact of solvent system, catalyst type, and reaction time and temperature. We also discuss unexpected solubility changes in the obtained products during storage, and propose a cause and remedy of that instability.

4.2 Experimental section

4.2.1 Materials

Cellulose acetate propionate (CAP-504-0.2), cellulose acetate butyrate (CAB-553-0.4), and cellulose acetate (CA-320S) were from Eastman Chemical. Triethylamine (Et_3N) and 1,3-dimethyl-2-imidazolidinone (DMI) were purchased from Acros Organics. Toluene, N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMAc), methyl ethyl ketone (MEK) and dichloromethane were purchased from Fisher Scientific. Anhydrous tetrahydrofuran, acrylic acid, butylhydroxytoluene (BHT) and Grubbs' catalysts were purchased from Sigma Aldrich. Diethylene glycol monovinyl ether was purchased from TCI. 10-Undecenoyl chloride was purchased from Pfaltz & Bauer Inc. DMAc and DMI were dried over 4 Å molecular sieves and MEK was dried by refluxing over potassium carbonate before use. All other purchased reagents were used as received.

4.2.2 Measurements

^{1}H NMR spectra were acquired on INOVA 400 or Bruker Avance 500 spectrometers operating at 400 or 500 MHz. Samples were analyzed as solutions in $CHCl_3$ or DMSO-d6 (ca. 10 mg/mL) at 25 ℃ in standard 5 mm o.d. tubes. Three drops of trifluoroacetic acid were added to shift the water peak in DMSO-d6 downfield from the spectral region of interest. ^{13}C NMR and ^{1}H-^{13}C HSQC spectra were obtained on a Bruker Avance 500 MHz spectrometer with a minimum of 5000 scans in DMSO-d6 (ca. 50 mg/mL) at 80 ℃. To obtain the T_g values of the cellulosic polymers, modulated DSC was performed on a TA Instruments Q2000 apparatus. Dry powders (ca. 5 mg) were loaded in Tzero^{TM} aluminum pans. Each sample was equilibrated at -50 or -20 ℃. The scanning conditions were set as follows: the underlying ramp heating rate was 7 ℃, the oscillation amplitude was ±1 ℃, and oscillation period was 40 s. FTIR spectra were obtained on a Nicolet 8700 instrument. Size exclusion chromatography (SEC) was performed in HPLC grade THF at 40 ℃ at flow rate 1 mL/min using a Waters size exclusion chromatograph equipped with an autosampler, three in-line 5 μm PLgel Mixed-C columns, and a Waters 410 refractive index (RI) detector operating at 880 nm, which was programmed to a polystyrene calibration curve. Cellulose ester solubility was tested by adding ca. 10 mg of sample into 2 mL each of various solvents. Each mixture was subjected to vortex mixing for 5-10 min at room temperature, and then solubility was judged by visual examination.

4.2.3 Preparation of cellulose esters with terminally olefinic sidechains

Preparation of Cellulose Acetate 10-Undecenoate (CAUn). CA-320S (1.00 g, 4.19 mmol/AGU) was dissolved in DMI (30 mL), and the solution was heated to 90 ℃ with mechanical stirring under N_2. Triethylamine (1.29 mL, 9.22 mmol, 2.2 equiv.; or 3.20 mL, 23.0 mmol, 5.5 equiv.) was added; a condenser was used to avoid evaporative loss of the base catalyst. 10-Undecenoyl chloride (1.70 g, 8.36 mmol, 2.0 equiv.; or 4.25 g, 20.95 mmol, 5.0 equiv.) was added dropwise and allowed to react at 90 ℃ for 20 h. The reaction mixture was then filtered, and the filtrate was precipitated in 300 mL 50:50 water/ethyl alcohol. The precipitate was redissolved in a minimal amount of CH_2Cl_2 and reprecipitated in hexane. The product was washed with hexane and dried under vacuum at 40 ℃. ^{1}H NMR ($CDCl_3$): 1.22 (br s, $COCH_2CH_2C\underline{H_2}C\underline{H_2}C\underline{H_2}C\underline{H_2}CH_2CH_2CH_2CH=CH_2$), 1.33 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2C\underline{H_2}CH_2CH=CH_2$), 1.53 (br s, $COCH_2C\underline{H_2}CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$), 1.88-2.03(m,

$COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$, and $COC\underline{H}_3$), 2.26 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CH_2$), 3.25-5.24 (m, cellulose backbone), 4.85-4.94 (q, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}_2$), 5.75 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CH_2$). ^{13}C NMR ($CDCl_3$): 20.4 ($CO\underline{C}H_3$), 24.8 ($COCH_2\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$), 28.8 ($COCH_2CH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2CH_2CH=CH_2$), 33.6 ($CO\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2\underline{C}H_2CH=CH_2$), 114.1 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=\underline{C}H_2$), 139.0 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2\underline{C}H=CH_2$), 168.9-173.1 (C=O), 62.2 (C-6), 72.0-76.4 (C2, C3, C5), 82.3 (C-4), 100.7 (C-1). For the batch with 2.0 equivalents/AGU 10-undeconoyl chloride, degrees of substitution (DS) by ^{1}H NMR: DS(10-undecenoate) (DS(Un)) 0.67, DS(acetate) (DS(Ac)) 1.88; yield: 93.6%. For the batch with 5.0 equivalents/AGU 10-undeconoyl chloride, DS by ^{1}H NMR: DS(Un) 1.28, DS(Ac) 1.86; yield: 90.7%.

Example Procedure for the Preparation of Cellulose Acetate Propionate 10-Undecenoate (CAPUn). CAP-504-0.2 (1.00 g, 1.78 mmol/AGU) was dissolved in MEK (20 mL), and the solution was heated to 60 ℃ with magnetic stirring under N_2. After the addition of triethylamine (0.54 mL, 1.96 mmol, 1.1 equiv.), 10-undecenoyl chloride (0.72 g, 3.56 mmol, 1.0 equiv.) was added dropwise, and the mixture was stirred for 20 h at 60 ℃. After filtration to remove triethylammonium chloride, the filtrate was precipitated into 300 mL 50:50 water/ethyl alcohol. The product was redissolved in CH_2Cl_2, reprecipitated in hexane and dried under vacuum at 40 ℃.

A similar procedure was followed for the preparation of CAB 10-undecenoate (CABUn).

CABUn. ^{1}H NMR ($CDCl_3$): 0.87-0.97 (m, $COCH_2CH_2C\underline{H}_3$), 1.27-1.35 (m, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH=CH_2$, 1.50-1.67 (m, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$, and $COCH_2C\underline{H}_2CH_3$), 2.03-2.32 (m, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CH_2$, $COC\underline{H}_2CH_2CH_3$, and $COC\underline{H}_3$), 3.25-5.24 (m, cellulose backbone), 4.89-4.99 (q, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}_2$), 5.78 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CH_2$). DS(Un) by ^{1}H NMR: 0.36. Yield: 98.0%.

CAPUn. ^{1}H NMR ($CDCl_3$): 0.99-1.18 (m, $COCH_2C\underline{H}_3$), 1.27-1.35 (m, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH=CH_2$), 1.55-1.63 (m, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$), 2.01(m,

$COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$), 2.15-2.36 (m, $COC\underline{H}_3$, $COC\underline{H}_2CH_3$, and $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CH_2$), 3.25-5.24 (m, cellulose backbone), 4.89-4.99 (q, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}_2$), 5.78 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CH_2$). DS(Un) by ^{1}H NMR: 0.51. Yield: 94.2%.

4.2.4 Olefin cross-metathesis

General Procedure for Olefin Metathesis of Cellulose 10-Undecenoate derivatives with Acrylic Acid as Solvent and Reagent. To a flask charged with cellulose 10-undecenoate derivative (100 mg, 1.0 equiv. of olefin), 5 mg BHT and 3 mL acrylic acid were added. After the reagents were completely dissolved, Hoveyda-Grubbs Catalyst 2nd Generation (0.03 equiv in THF) was added via syringe. After stirring for 1 h under N_2 at 30 ℃, the reaction was stopped by adding 1-2 drops of diethylene glycol monovinyl ether. The product was precipitated in H_2O and sufficiently washed by H_2O before being dried under vacuum at 40 ℃

General Procedure for Olefin Metathesis of Cellulose 10-Undecenoate derivatives with Acrylic Acid in THF. To a flask charged with cellulose 10-undecenoate derivatives (100 mg, 1.0 equiv. olefin), 5 mg BHT and 3 mL THF were added. After the reagents were completely dissolved, acrylic acid (20 equiv) was added followed by the addition of Hoveyda-Grubbs Catalyst 2nd Generation (0.03 equiv in THF) via syringe. After stirring for 1 h under N_2 at 30 ℃, the reaction was stopped by adding 1-2 drops of diethylene glycol monovinyl ether. The product was precipitated in H_2O and sufficiently washed by H_2O before being dried under vacuum at 40 ℃.

Similar procedures were followed for the metathesis reaction performed in other solvent systems.

Cellulose Acetate Monododec-10-endioate (CADod)

^{1}H NMR (DMSO-d_6): 1.23 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2CH=CHCOOH$), 1.38 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2CH=CHCOOH$), 1.50 (br. s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOH$), 1.86-2.14(m, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOH$, and $COC\underline{H}_3$), 2.28 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CHCOOH$), 2.75-5.25 (m, cellulose backbone), 5.68(br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOH$, *Z* configuration), 5.74 (d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOH$, *E* configuration), 6.19 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOH$, *Z* configuration), 6.80 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOH$, *E* configuration). ^{13}C NMR (DMSO-d_6): 20.7 ($CO\underline{C}H_3$), 24.8 ($COCH_2\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOH$), 28.0

($COCH_2CH_2CH_2CH_2CH_2CH_2\underline{C}H_2CH_2CH=CHCOOH$), 29.0
($COCH_2CH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2CH_2CH_2CH=CHCOOH$), 31.8
($COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOH$), 33.8
($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CHCOOH$),
122.4($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=\underline{C}HCOOH$),
149.0($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=\underline{C}HCOOH$),
167.4($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH\underline{C}OOH$), 169.1-173.3 (C=O), 63.0 (C-6), 72.0-76.4 (C2, C3, C5), 80.4 (C-4), 100.0 (C-1). For cellulose acetate 10-undecenoate with DS of 0.67, conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: 16.7, yield: 92.6%. For cellulose acetate 10-undecenoate with DS of 1.28, conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: 4.0, yield: 71.0%.

Cellulose Acetate Butyrate Monododec-2-endioate (CABDod)

^{1}H NMR (DMSO-d_6): 0.83-0.91 (m, $COCH_2CH_2C\underline{H}_3$), 1.26 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2CH=CHCOOH$), 1.33-1.67 (m, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2CH=CHCOOH$, $COCH_2C\underline{H}_2CH_3$), 1.99-2.40(m, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CHCOOH$, $COC\underline{H}_2CH_2CH_3$, and $COC\underline{H}_3$), 2.75-5.25 (m, cellulose backbone), 5.68(br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOH$, *Z* configuration), 5.73 (d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOH$, *E* configuration), 6.19 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOH$, *Z* configuration), 6.79 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOH$, *E* configuration). Conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: ~5, yield: 85.2%.

Cellulose Acetate Propionate monododec-2-endioate (CAPDod)

^{1}H NMR (DMSO-d_6): 0.93-1.04 (m, $COCH_2C\underline{H}_3$), 1.24 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2CH=CHCOOH$), 1.37 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2CH=CHCOOH$), 1.48 (br. s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOH$), 2.13-2.29 (m, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CHCOOH$, $COC\underline{H}2CH3$, and $COC\underline{H}_3$), 2.75-5.25 (m, cellulose backbone), 5.68(br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOH$, *Z* configuration), 5.72 (d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOH$, *E* configuration), 6.18 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOH$, *Z* configuration), 6.79 (m,

$COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH$=CHCOOH, *E* configuration). Conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: 20.0, yield: 95.2%.

4.2.5 Alkaline hydrolysis

About 0.5 g of cellulose ester and 25 mg BHT were placed in a 100 mL flask. Aqueous ethanol (20 mL, 70%) was then added and the mixture was occasionally stirred for 1 h at room temperature. Aqueous sodium hydroxide (20 mL, 0.5 M) was then added to the flask. The flask was sealed and kept at 50 ℃ for 24 h with constant stirring. After filtration, the filtrate was extracted several times by CH_2Cl_2. The organic extracts were collected and concentrated by rotary evaporation, followed by drying in vacuum oven at 40 ℃.

4.3 Results and discussion

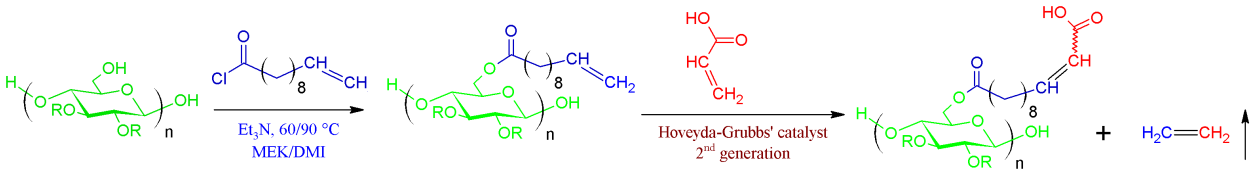


Figure 4.2. General two-step synthetic method for cellulose ω-carboxyalkanoate derivatives. (Note that structures are not meant to imply regiospecificity; the particular positions of substitution are shown in all Schemes only for convenience of depiction.)

Terminally-unsaturated fatty acids are type I olefins in Grubbs' classification, prone to rapid homodimerization. Many researchers have studied CM of such small molecules with methyl acrylate[30,31], acrylonitrile[31], allyl chloride[32] and alkynes[33], leading to a variety of end-functionalized fatty acids. In fact, terminally-unsaturated fatty acids can be made by cross-metathesis between unsaturated fatty acids from natural oils and ethylene[34]. We prepared esters of cellulose containing long chain esters with terminal unsaturation (CA-, CAB-, CAP-10-undecenoate) by methods similar to those previously used for synthesis of esters of cellulose with saturated long chain acids,[16-18,35,36] in particular by esterifying commercially available cellulose esters with 10-undecenoyl chloride in the presence of Et_3N (**Table 4.2**). DS(10-undecenoate) (DS(Un)) of adducts with CA, CAB, and CAP was kept mostly in the range 0.4 to 0.7 in order to obtain derivatives with relatively high glass-transition temperature (T_g)[37] values, advantageous for minimizing drug mobility and thus crystallization in ASD formulations[38]. One

higher DS(Un) sample (DS 1.28) was prepared in order to examine whether the CM reaction would still be effective at higher olefin densities.

Table 4.2. Synthesis of cellulose ester undecenoates

Sample	Starting cellulose ester	Solvent	Molar ratio[a]	Temp. (°C)	Product	DS (Un)[b]	DS (other)[c]
1	CA-320S	DMI	2.0	90	CAUn067	0.67	Ac 1.82, 1.88[b]
2	CA320S	DMI	5.0	90	CAUn128	1.28	Ac 1.82, 1.86[b]
3	CAB-553-0.4	MEK	1.0	60	CABUn036	0.37	Ac 0.14, Bu 1.99,
4	CAP-504-0.2	MEK	1.0	60	CAPUn051	0.51	Ac 0.04, Pr 2.09

[a]Mol 10-undecenoyl chloride per mol anhydroglucose unit. [b]Determined by ^{1}H-NMR. [c]Reported in a previous publication[16].

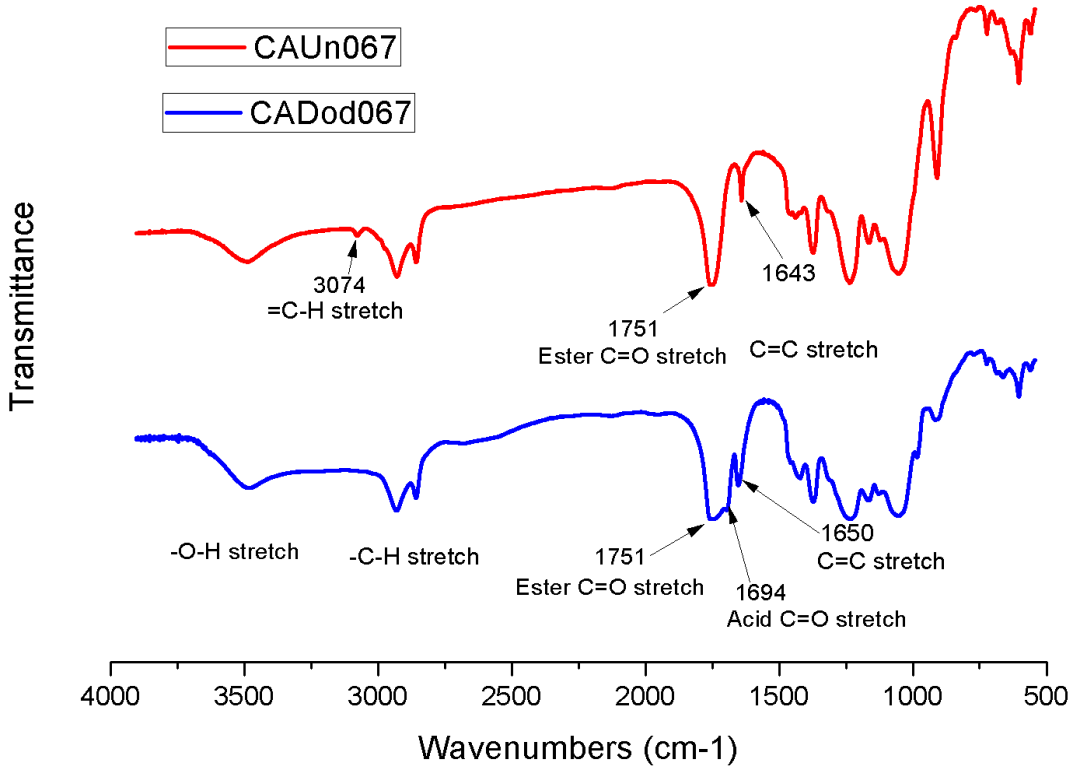


Figure 4.3. FTIR spectra of CAUn067 (**2**) and CADod067 (**7**).

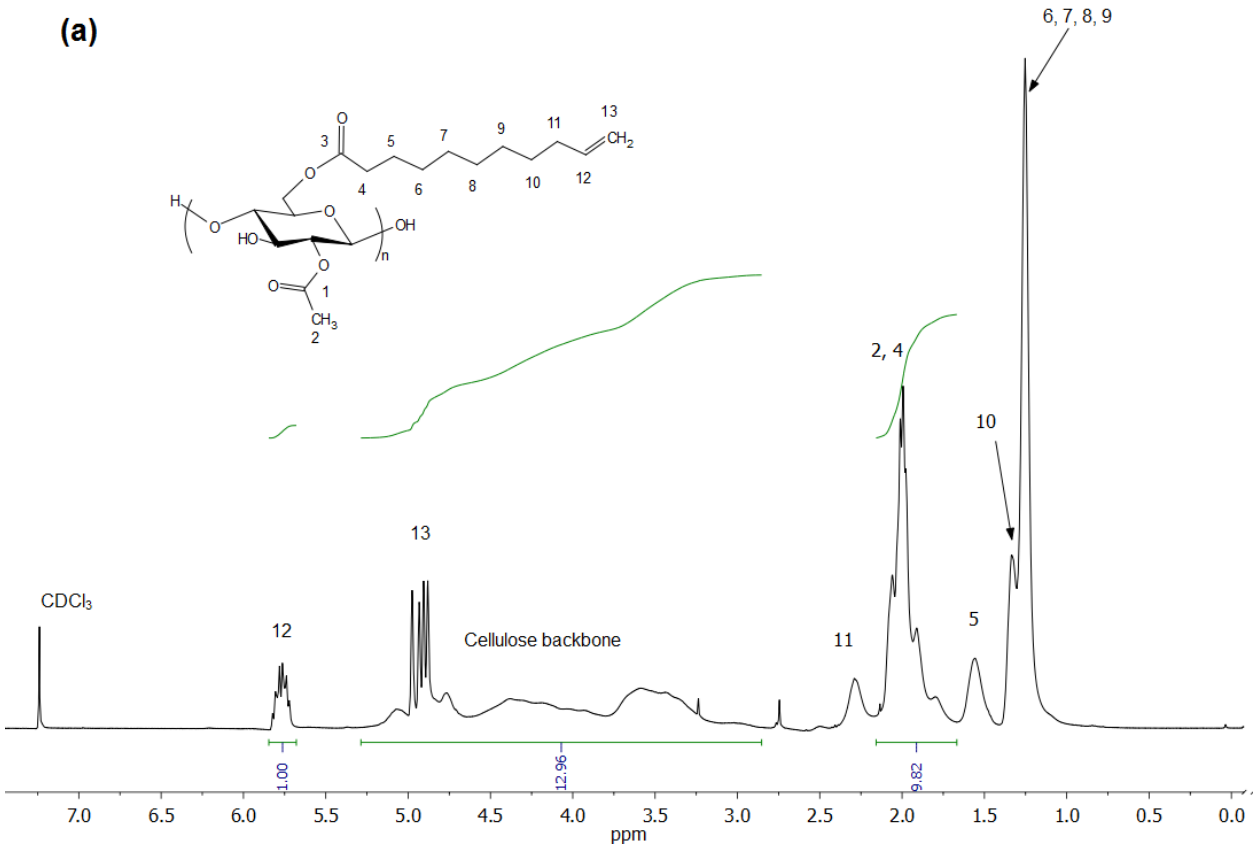
For successful synthesis of soluble, uncrosslinked cellulose w-carboxyalkanoates, we needed to maximize CM between the terminal olefin of the cellulose alkanoate undecenoate and acrylic acid, and suppress SM of both starting materials and products. We explored the impact of several factors known to be important in CM of small molecules, including catalyst type and loading, stoichiometry, and reaction time and temperature (**Table 4.3**). Grubbs' 2nd generation catalyst (**C2**) and Hoveyda-Grubbs' 2nd generation catalyst (**C3**) were compared using CA-U067 as starting material and acrylic acid as both reagent and solvent. Clearly **C3** was much more effective than **C2** (**Exp. 1, 2**) in this system. Catalyst loading also significantly affected CM conversion (characterized herein as the percent of the terminal olefin groups that underwent CM); 2 mol% **C3** afforded ~90 % conversion, while we were gratified to observe that 3 mol% catalyst loading gave complete conversion, even under very mild conditions (room temperature) and with rapid kinetics (complete in 30 min). Such mild conditions and short reaction times were encouraging for cases in which acrylic acid was used as both reactant and solvent, promising to minimize any acid-catalyzed solvolysis of glycosidic linkages. From the FTIR spectra (**Figure 4.3**, Supporting Information **S4.1 – S4.3**), we can see that the peak at 1643 cm^{-1}, which was assigned to the C=C stretch of the starting cellulose ester undecenoates, was shifted to 1650 cm^{-1} after CM. We also observed the appearance of a C=O stretch absorbance at 1694 cm^{-1} on the shoulder of the ester C=O stretch peak at 1751 cm^{-1}, further supporting the success of CM. Proton NMR was a useful tool for following the reaction (**Figure 4.4, S4.4, S4.5**), by following disappearance of the terminal olefin protons of the starting material at 4.90 and 5.75 ppm. New proton signals appeared at 5.73 and 6.80 ppm, which were assigned to protons of the α,β-unsaturated carboxylic acid in its *E* configuration. Correspondingly, the signals of the product olefin in the *Z* configuration were found at 5.68 and 6.19. ^{13}C NMR spectra (**Figure 4.5**) showed the complete disappearance of starting terminal olefin signals at 114 and 139 ppm, and the appearance of new peaks at 122 and 149 ppm, supporting the conclusion that 100% CM conversion had occurred. To further confirm the assignment of proton peaks of *E/Z* configuration, ^{1}H-^{13}C HSQC was performed (**S4.6**), which showed correlation of the carbon signal at 122 ppm with proton signals at 5.73 and 5.68 ppm, and correlation of the carbon signal at 149 ppm with proton signals at 6.80 and 6.19 ppm. Integration of the ^{1}H NMR spectrum showed that the *E* configuration was obtained in strong preference to the Z configuration, which is in consistent with previous reports[13,30,31]. However, the *E/Z* ratio for the CA product was

observed to vary from experiment to experiment; this phenomenon was also observed in reactions of CAB and CAP undecenoates. It should be noted that to whatever extent the E/Z mixture is an issue for a particular application, it could be alleviated by subsequently subjecting the product α,β-unsaturated carboxylic acids to reactions such as Michael addition[39] or hydrogenation[40] that would eliminate the double bond. We also observed highly selective cross-metathesis with CABUn036, CAPUn051and CAUn128 under similar conditions, also reaching 100% conversion (^{1}H NMR, **S4.4** and **S4.5**). Even in the case of CM of acrylic acid with CAUn128, which has nearly twice the olefin density of CAUn067, 100% CM was observed, with no sign of crosslinking.

Table 4.3. CM of cellulose 10-undecenoate derivatives with acrylic acid.

Sample	Start. mat.	Cat. type	Cat. Load. (mol%)	Solvent[a]	Mol. ratio[b]	Temp. (℃)	Time (h)	Conv.	Product	DS (Dod)[d]	DS (other)[d]
5	CAUn067	C2	2	AA	--	50	24	~0	--	--	--
6	CAUn067	C3	2	AA	--	50	24	~90	--	--	--
7	CAUn067	C3	3	AA	--	30/r.t.	0.5/1	~100	CADod067	0.77	Ac 1.77
8	CAUn128	C3	3	AA	--	30	1	~100	CADod128	1.32	Ac 1.80
9	CAUn067	C3	3	DCM	1:5	30	1	N. A.[c]	--	--	--
10	CAUn067	C3	3	DCM	1:20	30	1	~100	CADod067 in DCM	0.77	Ac 1.84
11	CAUn067	C3	3	$CHCl_3$	1:20	30	1	~90[b]	--	--	--
12	CAUn067	C3	3	THF	1:5	30	1	~90	--	--	--
13	CAUn067	C3	3	THF	1:20	30	1	~100	CADod067 in THF	0.72	Ac 1.82
14	CABUn036	C3	3	AA	--	r.t.	1	~100	CABDod036	0.38	Bu 2.00[e]
15	CAPUn051	C3	3	AA	--	r.t.	1	~100	CAPDod051	0.54	Pr 2.11[e]

[a]AA: acrylic acid; DCM: dichloromethane; THF: tetrahydronfuran. [b]mol of terminal double bond : mol of acrylic acid. [c]Gelation was observed during the reaction; [d]Determined by ^{1}H NMR; [e]Due to overlapping and relatively small values, DS (Ac) in CAB and CAP derivatives determined by NMR are not reliable.


(a)
6, 7, 8, 9
2, 4
10
13
CDCl3
12
Cellulose backbone
11
5
1.00
12.96
9.82
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

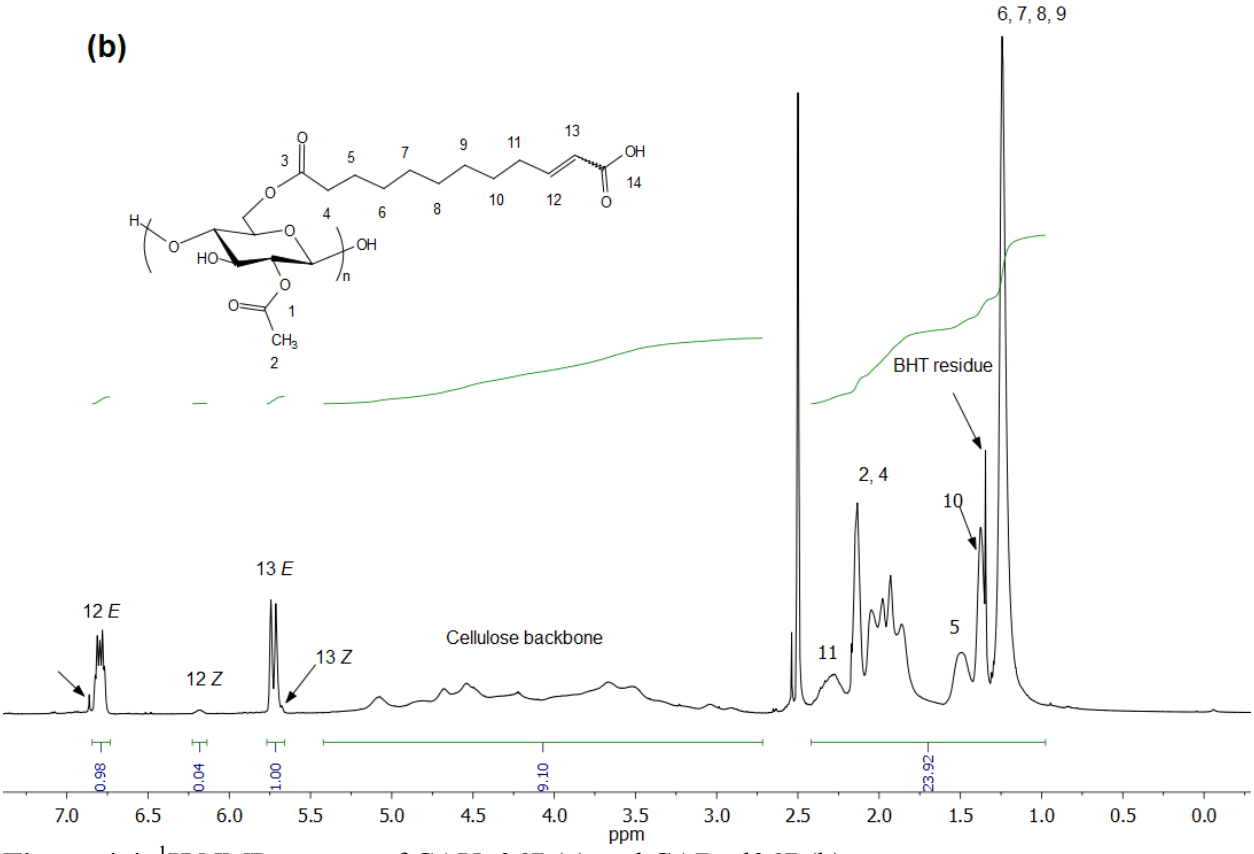
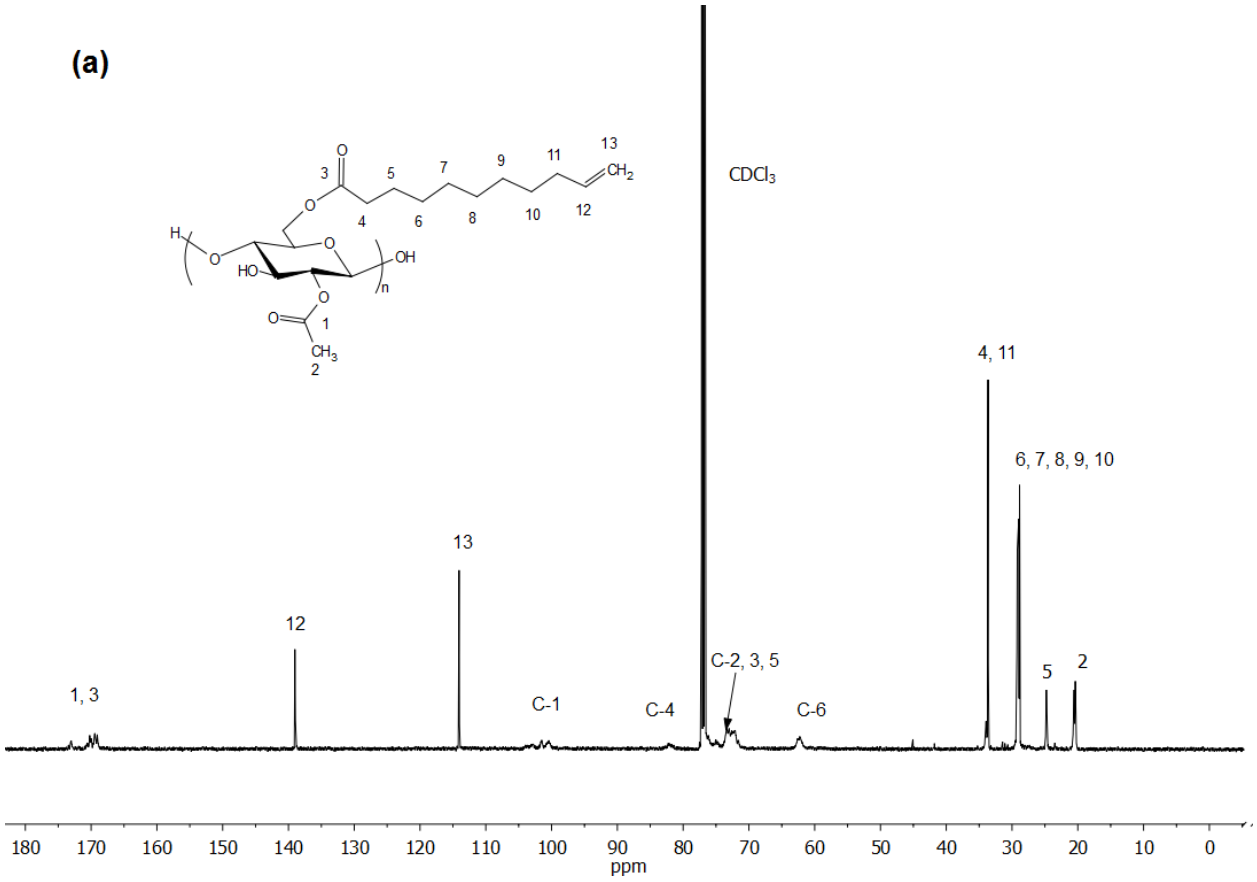


Figure 4.4. ^{1}H NMR spectra of CAUn067 (a) and CADod067 (b).


(a)
CDCl3
4, 11
6, 7, 8, 9, 10
13
12
C-2, 3, 5
5
2
1, 3
C-1
C-4
C-6
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

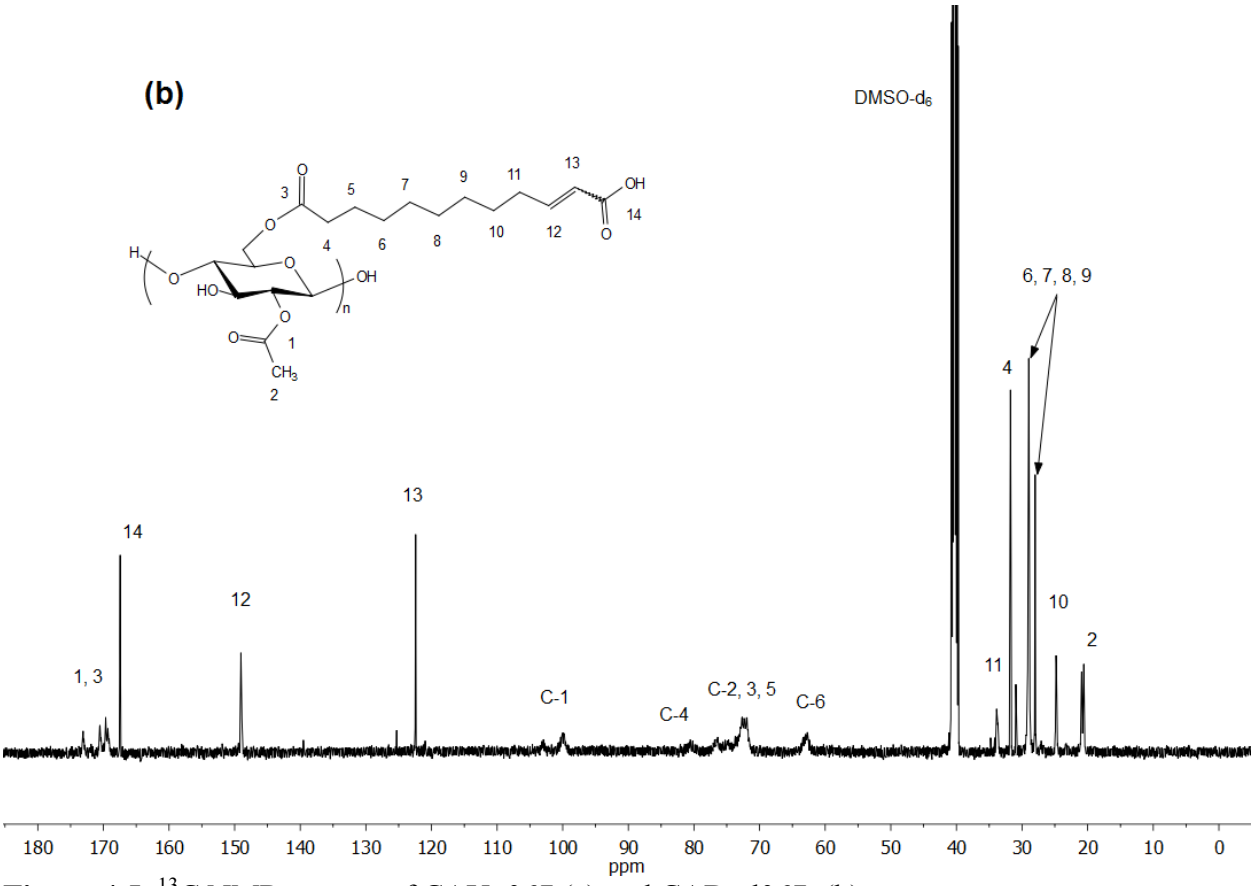


Figure 4.5. ^{13}C NMR spectra of CAUn067 (a) and CADod067 (b).

We were pleased with the success of the CM reaction in acrylic acid as reagent and solvent, but were watchful about two potential problems: 1) the possibility of acid-catalyzed solvolysis of the glycosidic linkages due to the preponderance of acrylic acid, and 2) the possibility that other terminal and electron-deficient olefins might be incompatible with acrylic acid solvent due to solubility or miscibility issues (e.g. acrylamide, unpublished results). For these reasons, other solvent systems were investigated using CAUn067 (**1**) as starting material. Dichloromethane, commonly used as an OM solvent, was investigated first. However, with an acrylic acid : terminal olefin ratio of 5:1 and all other conditions the same as those in acrylic acid solvent, gelation was observed at the end of the reaction, indicating the possibility that substantial intermolecular SM had occurred. Upon using THF as solvent and an acrylic acid : terminal olefin ratio of 5:1, incomplete CM (~90% conversion) was observed, though there was no gelation. Increasing the acrylic acid: olefin ratio to 20:1 afforded completely cross-metathesized products in either CH_2Cl_2 or THF. In contrast, incomplete CM and slight gelation were observed under the same conditions when $CHCl_3$ was used as solvent. The results obtained

in different solvents can be rationalized by a change in polymer solubility as a result of the CM reaction. Although the starting polymer was readily soluble in either CH_2Cl_2 or $CHCl_3$ **(Table 4.4)**, the acrylic acid CM product was no longer soluble in these solvents. While the polymer may not necessarily precipitate as the reaction proceeds, its decreased solubility may cause it to aggregate, increasing the likelihood of intra- and intermolecular SM. THF, on the other hand, is a good solvent for both the starting material and the final product. As a result, no precipitation, aggregation, or aggregation-induced SM is observed in THF.

Table 4.4. Solubility of cellulose 10-undecenoate derivatives and cellulose monododec-10-endioate derivatives in various solvents.

Sample	CH_2Cl_2	$CHCl_3$	EtOAc	iPrOH	THF	Acetone	DMSO	DMF
CAUn067 (**1**)	+	+	+	-	+	+	+	+
CADod067 (**7**)	-	-	-	-	+	+	+	+
CAUn128 (**2**)	+	+	+	-	+	+	+	+
CADod128 (**8**)	P	P	+	-	+	+	+	+
CABUn036 (**3**)	+	+	+	-	+	+	+	+
CABDod036 (**14**)	P	P	+	-	+	+	+	+
CAPUn051 (**4**)	+	+	+	-	+	+	+	+
CAPDod051 (**15**)	P	P	+	-	+	+	+	+

(+) soluble; (-) insoluble; (P) partially soluble

We used SEC to determine the degree of molecular weight change during the reaction (**Table 4.5**). For CABUn036 and CAPUn051 (**3** and **4**), the degree of polymerization (DP) decreased only very slightly after 1 h CM reaction in acrylic acid at either room temperature or 30 ℃, showing that the mild reaction conditions did preserve polymer molecular weight even in an acidic solvent. As for CAUn067 (**1**) and CAUn128 (**2**), higher starting DP and less polar ester substituents (acetate) make them it more susceptible to chain scission by acid hydrolysis[41]. With these acetate esters, approximately 50% loss of M_n was observed after CM reaction of **1** for 1h at 30 ℃ in acrylic acid solvent, although performing the reaction in THF preserved DP to some extent (30% loss of M_n). Dispersity (Đ) data from SEC chromatograms can be used to indicate whether or not significant intermolecular SM occurred. SEC data of CAP and CAP derivatives (**3**, **4**, **14** and **15** in **Table 4.5**) showed that chain scission was negligible during CM; these almost

unchanged Đ values strongly indicate that intermolecular SM was successfully suppressed. In the case of the cellulose acetate derivatives (**1**, **2**, **7**, **8** and **13** in **Table 4.5**), presumably acid-catalyzed chain scission complicates the interpretation of the SEC data, but certainly there is no positive indication of SM occurring with these derivatives.

The CM conditions are mild and there is no obvious solvolytic reagent present, but it is still of interest to confirm that the ester groups survived the reaction intact. ^{1}H NMR spectra clearly reveal DS (Dod), since the olefin signals and those of the backbone can be cleanly integrated and the ratio determined; no significant decrease in DS (Dod) occurs during CM. A slight increase of calculated DS (Dod) is attributed to the broadening of cellulose backbone peak due to the introduction of carboxylic acid group. The alkyl proton signals of acetyl, propionyl, and butyryl moieties overlap with those of the Dod group, complicating measurement of their DS ^{1}H NMR. For CA derivatives (e.g. CADod067), as DS (Dod) are known, we can calculate DS (Ac) by integrating and comparing the ratio of the olefin signals with the methyl and methylene signals. Similarly, DS (Bu) and DS (Pr) in CABDod036 and CAPDod051 respectively were determined by integrating and comparing the ratio of the olefin signals with the methyl signals of the acyl groups. The results (**Table 4.3**) showed that negligible hydrolysis of acetyl, butyryl and propionyl groups occurred during the olefin metathesis reaction.

Table 4.5. Molecular weight and T_g of the cellulose esters.

Sample	Mn (kDa)	DP	Đ	T_g (°C)
CA-320S	38.0[a]	151.4	NA	180[b]
CAUn067 (**1**)	36.8	100.3	1.98	--
CADod067 (**7**)	16.4	42.0	1.56	115
CADod067 in THF (**13**)	26.6	68.2	2.16	109
CAUn128 (**2**)	25.4	54.8	1.72	--
CADod128 (**8**)	19.9	38.3	1.36	-15, 94
CAB-553-04	20.0[a]/22.1	72.0	2.54	100[b]
CABUn036 (**3**)	24.8	64.4	2.24	--
CABDod036 (**14**)	23.4	57.8	2.25	93
CAP-504-02	15.0[a]/15.2	54.1	2.04	158[b]
CAPUn051 (**4**)	17.7	48.5	1.92	--
CAPDod051 (**15**)	16.4	42.3	1.86	81

[a]Data reported by supplier, versus polystyrene standards. [b]Data reported in previous publication[35].

Polymer glass transition temperature is an important parameter influencing the potential for its use as an ASD matrix. As the glassy state restricts drug mobility and resulting

crystallization, a polymer with T_g that is at least 50 ℃ higher than ambient temperature is preferred in ASD formulations. This keeps the formulation T_g above ambient temperature in spite of the plasticizing effects of both drug and atmospheric moisture. For the long chain CM product synthesized herein, only very weak thermal transitions were observed by standard DSC methods. We employed modulated DSC to give sharper transitions (**Table 4.5**, **S4.7**). Although the T_g values of the CM products decreased 10 to 80 ℃ compared with those of the starting cellulose esters, they still remained at least 50 ℃ higher than room temperature. As expected, the higher DS of long side chain produced a lower T_g value for sample **8** than that for **7**. The high DS CM product **8** also displayed an extra low temperature transition at -15 ℃, which we attribute to cooperative motion of the long side chains by analogy to results from other studies of long chain cellulose esters[35,42].

During the investigation of the CM reaction, we observed an intriguing and disturbing phenomenon. CM products lost solubility during storage, and the polymer with higher DS of α,β-unsaturated carboxylic acid lost solubility faster than those with lower DS. Moreover, dissolving the originally soluble polymer in THF and then precipitating it in hexane in some cases generated insoluble product immediately. Apparently, certain reaction(s) caused rapid, substantial crosslinking, and the crosslinking had something to do with the α,β-unsaturated carboxylic acid groups. We were concerned about the possibility of continuing, secondary cross-metathesis of the pendant α,β-unsaturated carboxylic acid groups of the product, which could be caused by residual catalyst. To exclude this possibility, we added diethylene glycol monovinyl ether at the end of metathesis reactions; diethylene glycol monovinyl not only can quench the CM reaction, but the glycol tail makes the catalyst water-soluble so that it can be easily removed by washing the product with water.[43] The addition of diethylene glycol monovinyl ether did not mitigate the solubility issue, showing that CM was not the cause of the insolubility. We then turned our attention to two other possible undesired reactions of the CM products, Michael reaction or free radical polymerization. We thought that Michael addition to the α,β-unsaturated carboxylic acid groups was less likely, due to the absence of strong base catalyst, and the relatively low reactivity of the possible Michael donors (-OH or methylene groups). We therefore examined whether the crosslinking was caused by a free radical mechanism. We carried out experiments in which 3,5-di-*tert*-4-butylhydroxytoluene (BHT), a commonly used antioxidant capable of scavenging free radicals, was added before the reaction. As BHT is not

soluble in water, the majority of it would remain in the polymer upon addition of the reaction mixture to water to precipitate the product. The presence of BHT would be expected to suppress the generation of free radicals during reaction, isolation, and product storage. Indeed, the BHT-containing products remained soluble after two months storage at room temperature. Furthermore, dissolution of these products in THF and precipitation in hexane afforded products that were still readily soluble in THF. Prevention of crosslinking by BHT addition strongly supports the notion that the cross-linking and solubility loss was caused by a free radical mechanism, while providing a useful means to avoid the problem.

To further confirm this hypothesis, we performed alkaline hydrolysis of the ester groups of metathesis products according to a previously reported method[44]. After acidification, long chain acids were extracted into CH_2Cl_2, and then the CH_2Cl_2 extract was washed extensively by water. A suitable amount of BHT was added to prevent coupling during this process. ^{1}H-NMR was performed on the acid products collected from samples with and without solubility problems. While samples without solubility problems afforded an acid product with a spectrum identical with undec-2-enedioic acid, those with solubility problems afforded acids with an extra peak at 0.79 ppm in the proton spectrum, indicating the occurrence of dimerization (**Figure 4.6**, **S4.8**, and **S4.9**).

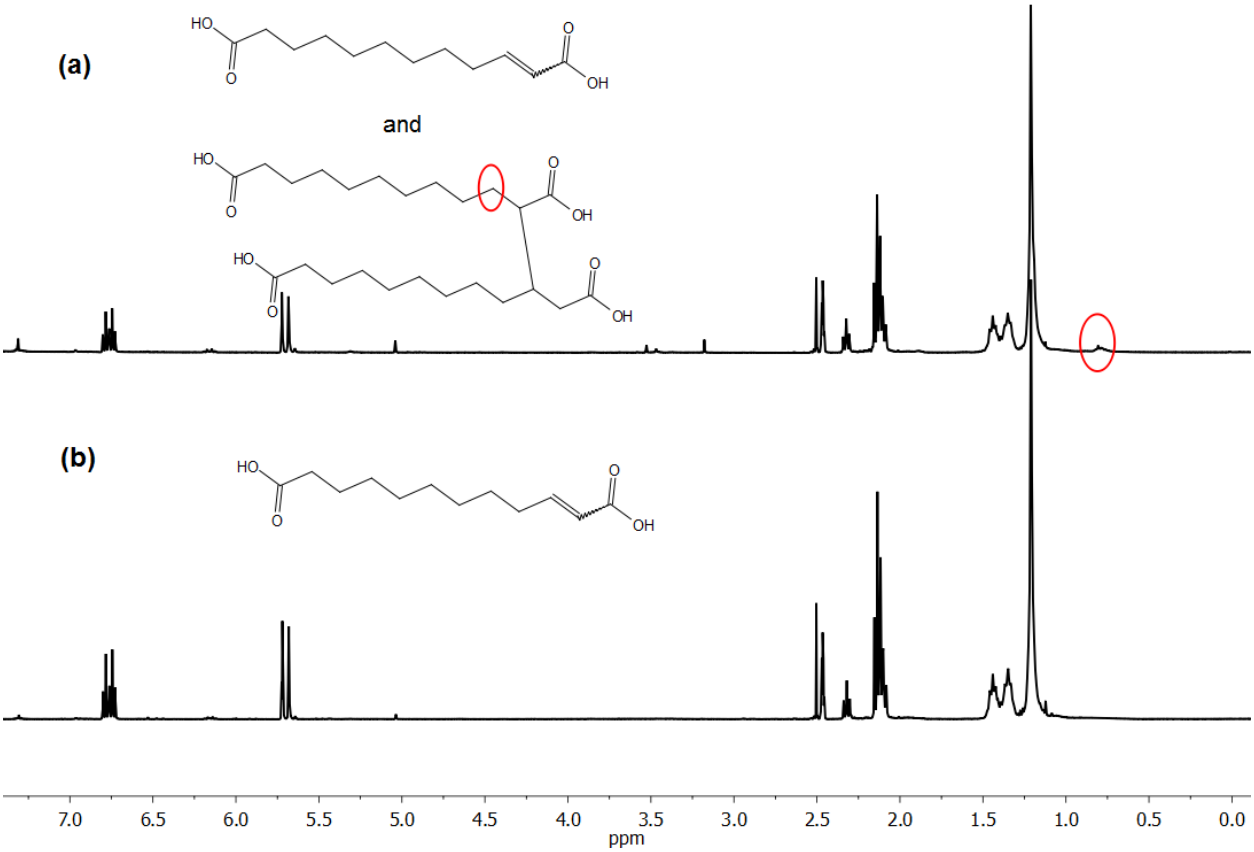


Figure 4.6. ^{1}H NMR spectra of alkaline hydrolysis products of cellulose esters having (a) and not having (b) solubility problems.

The synthesis of long chain α,β-unsaturated carbonyl derivatives via olefin metathesis has been the topic of several studies[10,30,45]. Recently, de Espinosa et al.[46,47] reported side-chain modification of poly(2-oxazoline) employing 10-undecenoyl as the side chain, via cross-metathesis with acrylates. However, none of these studies have mentioned the crosslinking problem that we report herein. Our results may provide a way to understand and avoid such crosslinking, while retaining the possibility that the double bond can be preserved should it be desirable to do so.

4.4 Conclusions

We report herein the first general procedure for the synthesis of cellulose ω-carboxyesters via olefin cross-metathesis. Previous literature precedent had been quite discouraging, in which self-metathesis dominated and insoluble, crosslinked products were obtained from similar cellulose derivatives. In contrast, we describe conditions in which fully cross-metathesized products were obtained by reacting cellulose esters bearing terminal olefins, in particular

cellulose alkanoate undecenoates, with acrylic acid as solvent and reagent, in THF, or in dichloromethane, employing the Hoveyda-Grubbs' 2nd generation catalyst. Crosslinking induced by intermolecular self-metathesis was avoided by using an excess of acrylic acid. While soluble products were obtained initially, loss of solubility during storage was observed and was attributed to oligomerization of the pendant α,β-unsaturated carboxylic acid groups by a free radical mechanism. We were able to suppress this free radical oligomerization and produce soluble products by addition of free radical scavengers such as BHT, thereby also creating the potential to preserve the double bond where this is desirable. This method allows for rapid, simple, versatile, highly efficient synthesis of cellulose w-carboxyalkanoates under mild conditions. There is every reason to expect that this new method will be applicable to other olefin partners, and that other polysaccharides containing terminal olefin groups will also be effective substrates for the reaction. One can imagine that a wide variety of CM partners such as acrylates and acrylamides might also be feasible for this strategy. Therefore the significance of these results is twofold: 1) it provides a pathway to new families of polysaccharide derivatives with different functional groups, which can be used for structure/property studies aimed at developing polymers for drug delivery and other demanding applications; 2) this strategy makes it possible to synthesize a series of polymer derivatives with identical Mw, DS, substitution pattern, and monosaccharide sequence, differing only in the terminal, pendant functional groups, enabling clean and enlightening investigations of structure-activity relationships.

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Chapter 5: Olefin cross-metathesis, a mild, modular approach to functionalized cellulose esters

(Adapted from Meng, X.; Matson J. B. and Edgar, K. J. Olefin cross-metathesis, a mild, modular approach to functionalized cellulose esters. Polym. Chem. 2014, 5 (24), 7021-7033)

Abstract:

Olefin cross-metathesis has been demonstrated to be a modular pathway for synthesis of a series of functionalized cellulose esters. As a proof of concept, cellulose acetate was acylated with two terminally olefinic acid chlorides, pent-4-enoyl chloride and undec-10-enoyl chloride, providing olefin-terminated cellulose esters with different side-chain lengths. These ω-unsaturated cellulose esters were then reacted with a variety of cross-metathesis partners, including acrylic acid, methyl acrylate, 2-hydroxyethyl acrylate, poly(ethylene glycol) methyl ether acrylate, and allyl alcohols, using Hoveyda-Grubbs' 2nd generation catalyst. Complete conversion to cross-metathesis products was achieved in reactions with acrylic acid or acrylates using 3-5 mol% catalyst at 40 ºC within 1 h. We further demonstrate successful hydrogenation of these α,β-unsaturated esters and acids, thereby eliminating the potential for radical-induced crosslinking during storage.

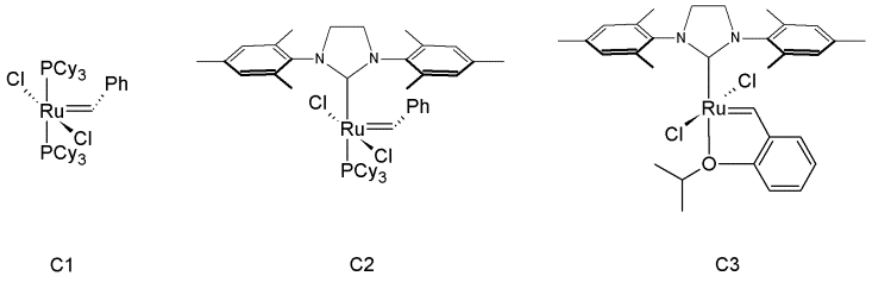
5.1 Introduction

Derivatives of cellulose and other polysaccharides are important components in a broad range of applications including drug delivery[1], automobile coatings[2], antimicrobials[3,4], and biomedical engineering[5-7]. Polysaccharide chemists use chemical modification to enhance processability and to tune polysaccharide properties to meet the requirements of specific applications. It is particularly important to modify cellulose, which is abundant, renewable, and non-toxic, but in its native state has extremely poor solubility and cannot be melt-processed. Cellulose derivatization can reduce interchain hydrogen bonding and crystallinity as a remedy for poor organic and water solubility, and can tailor viscoelastic properties,[8] thermal properties, and most importantly add new functional groups to the cellulosic backbone. The ability to broadly and selectively modify cellulose (or other polysaccharides) chemically can not only

convey the ability to change physicochemical properties; appending new functional groups can also open doors to various valuable applications. Functionalities including carboxylic acid, hydroxyl[9], amino,[10,11] and many others[12,13] have been used to enhance the performance of polysaccharides. For example, we have shown that synthetic methods allowing attachment of ω-carboxyalkanoate functionality to cellulose impart the capability for pH-controlled drug release, and for superior performance in generating supersaturated drug solutions from amorphous solid dispersions, due in part to enhanced specific interactions with drug molecules.[14,15] Others have pursued chemistries that permit the attachment of "tethers" to the polysaccharide, providing reactive sites (e.g. alcohol groups) that are distant from the main polysaccharide chain and hence more reactive, for easy attachment of targeting and other functional moieties[16-19].

To date polysaccharide chemistry and in particular cellulose chemistry has depended heavily on classical methods like esterification[11,20] and etherification[21] for appending functional groups, and indeed virtually all of the important commercial cellulose derivatives are made by either (or combinations) of these methods. While such methods are very useful and have led to entire industries based on the resulting derivatives of renewable cellulose, they are limited in scope. Typically esterification involves strongly acidic catalysts that are incompatible with sensitive functional groups on either cellulose or the acylating reagent, and esterification is also incompatible with difunctional reagents that could crosslink the product. Etherification typically involves strongly basic conditions (NaOH) in an aqueous environment, and so is incompatible with base-sensitive moieties and may be incompatible with reagents that do not possess substantial water solubility. Clearly there is a need to expand the toolkit for those seeking to make functional materials from sustainable polysaccharides; either by identifying new reactions that are mild, broadly useful, flexible, and ideally modular, or finding ways to extend the utility of conventional esterification and etherification to encompass such features. In this context we define "modular" polysaccharide reactions as those which permit the construction of a variety of functionalized moieties via small molecule chemistry, each of which can be attached to the polysaccharide using a reaction that is mild, high-yielding, efficient and dependable; in other words, a reaction bearing considerable similarity to "click" reactions. We will expand upon the differences in our chemistry from modern definitions of polymer click reactions[22] in the Conclusions section.

Click chemistry[23], first discovered by the Sharpless group, has enabled the rapid synthesis of molecules with diverse functional appendages. Among these powerful concerted reactions, the azide-alkyne Huisgen cycloaddition[24-27] and the thiol-ene click reaction[19,28-30] have been used a number of times in polysaccharide chemistry to prepare a variety of functionalized derivatives. These strategies are modular functionalization methods for cellulose derivatives that can enable useful structure-function relationship studies. These methods require use of odorous, toxic, and/or potentially energetic reagents, and necessitate introduction of one or more heteroatom functions (e.g. sulfide or 1,2,3-triazole), limiting the utility of these reactions.[31] They are valuable however, and the concepts[23] of being modular, wide in scope, providing high yields, and generating only inoffensive by-products can guide our explorations of alternative polysaccharide functionalization pathways.



C1 C2 C3

Figure 5.1. Commonly used Grubbs' catalysts for olefin metathesis.

Olefin cross-metathesis (CM) promises to fulfill the abovementioned criteria in polysaccharide derivative synthesis.[32] Grubbs' rules[33] predict that CM can be selective if two partners of differing reactivity are used; for example a type I olefin (e.g. reactive terminal olefin) and a type II or III olefin (e.g. less electron-rich acrylate); results also depend on the catalyst (**Figure 5.1**) used[33]. Driven by the loss of the volatile ethylene co-product, full conversion and high yields sometimes can be achieved. Recently, our group published an initial study showing that CM may be a powerful tool for the synthesis of soluble, discrete cellulose ω-carboxyesters.[34] This class of polysaccharide derivatives has been shown to have high promise for enhancing drug bioavailability by creating supersaturated drug solutions via amorphous solid dispersion[35,36]. These new derivatives were synthesized by CM between cellulose bearing olefin-terminated ester substituents (undec-10-enoate) and acrylic acid employing Hoveyda-Grubbs' 2nd generation catalyst. This first-ever demonstration of successful CM in polysaccharide chemistry (there were a few previous reports of self-metathesis of cellulose derivatives[37,38], and CM of

other olefin-functionalized polymers such as poly(oxazolines)[39]) hinted at its potential. CM of polysaccharides with differently functionalized partners may be a flexible way to functionalize cellulose and other polysaccharides in a modular manner to efficiently diversify the polysaccharide derivative family. The strategy is to attach a "handle" for CM using relatively conventional esterification chemistry, then carry out modular CM reactions to attach variously functionalized partners to cellulose, exploiting this handle (Fig. 2).

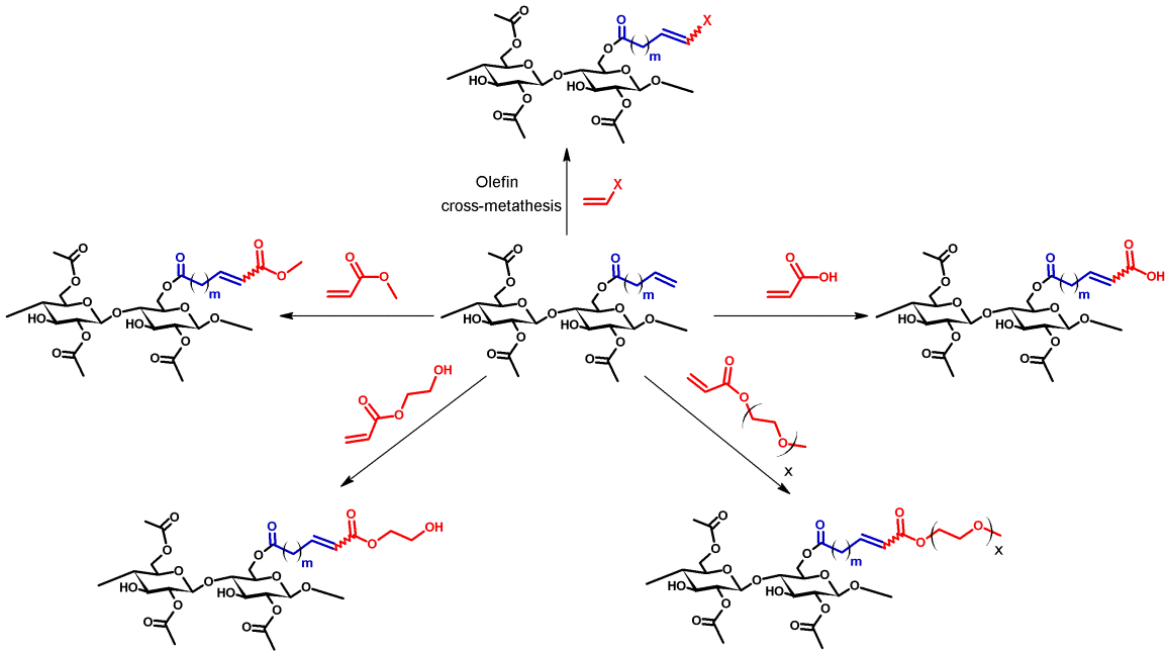


Figure 5.2. General scheme of olefin CM between terminally olefinic cellulose acetate and different CM partners.

We report herein exploration of such a modular CM approach for synthesis of a group of cellulose derivatives, particularly acrylate esters that would potentially be extremely useful for attaching additional functionality via the pendent ester group. We explore and discuss the scope of CM for cellulose ester functionalization. We provide initial tests of our hypothesis that olefin CM may be a general method for cellulose functionalization and take an important step towards utilization of this chemistry as a flexible, efficient modular strategy for preparation of polysaccharide derivatives that otherwise might remain inaccessible.

5.2 Experimental

5.2.1 Materials and instruments

Cellulose acetate (CA-320S, Mn ~ 38.0 kDa, DP ~ 151, DS(Ac) ~ 1.82 (data provided by supplier)) was from Eastman Chemical. Triethylamine and 1,3-dimethyl-2-imidazolidinone (DMI) were purchased from Acros Organics. Anhydrous tetrahydrofuran, acrylic acid, methyl acrylate, 2-hydroxyethyl acrylate, poly(ethylene glycol) methyl ether acrylate, allyl alcohol, 3-buten-2-ol, allylamine, 4-pentenoyl chloride, 10-undecenoyl chloride, ethyl vinyl ether, butylhydroxytoluene (BHT), palladium on carbon (10 wt% loading), Wilkinson's catalyst, Crabtree's catalyst, and Hoveyda-Grubbs' 2nd generatation catalyst were purchased from Sigma Aldrich. Diethylene glycol monovinyl ether was purchased from TCI. DMI were dried over 4 Å molecular sieves before use. All other purchased reagents were used as received. The high pressure reactor used in hydrogenation was mini bench top reactor 4560 purchased from Parr Instrument Company.

5.2.2 Measurements

^{1}H NMR spectra were acquired on a Bruker Avance 500 spectrometer operating at 500 MHz. Samples were analyzed as solutions in $CDCl_3$ or DMSO-d6 (ca. 10 mg/mL) at 25°C in standard 5 mm o.d. tubes. Three drops of trifluoroacetic acid were added to shift the water peak in DMSO-d6 downfield from the spectral region of interest. To obtain the T_g values of the cellulosic polymers, DSC was performed on a TA Instruments Q2000 apparatus using heat/cool/heat mode. Dry powders (ca. 5 mg) were loaded in Tzero^{TM} aluminum pans. The scanning conditions were set as follows: each sample was equilibrated at 35°C, and then heated to 150° at 20°C/min. The sample was then cooled at 100 °C/min to −50°C. During the second heating cycle the sample was heated to 200°C at 20°C/min. If the heat/cool/heat mode failed to give a clear transition, modulated DSC was performed as follows: each sample was equilibrated at -50°C, the underlying ramp heating rate was 7°C, the oscillation amplitude was ±1°C, and oscillation period was 40 s. FTIR spectra were obtained on a Nicolet 8700 instrument. Size exclusion chromatography (SEC), if not otherwise specified, was performed on Agilent 1260 Infinity Multi-Detector SEC using NMP with 0.05 M LiBr as the mobile phase (50 ºC) with 3 PLgel 10 μm mixed-B 300 × 7.5 mm columns in series. A system of multiple detectors connected in series was used for the analysis. A multi-angle laser light scattering (MALS)

detector (DAWN-HELEOS II, Wyatt Technology Corporation, Goleta, CA), operating at a wavelength of 658 nm, a viscometer detector (Viscostar, Wyatt Technology Corporation, Goleta, CA), and a refractive index detector operating at a wavelength of 658 nm (Optilab T-rEX, Wyatt Technology Corporation, Goleta, CA) provided online results. Data acquisition and analysis was conducted using Astra 6 software (Wyatt Technology Corporation, Goleta, CA). For several samples, SEC was performed in THF as mobile phase (40 °C) on Agilent 1260 Infinity Multi-Detector SEC. For both systems, monodisperse polystyrene standard (Mw~21k, Đ~1.02) was run first in every sample series for the purpose of calibration and confirmation.

5.2.3 Preparation of cellulose esters with terminally olefinic sidechains

Preparation of cellulose acetate 10-undecenoate (CA-Un, 2). CA-320S (**1**, 1.00 g, 4.19 mmol/AGU) was dissolved in DMI (30 mL), and the solution was heated to 90 °C with mechanical stirring under N_2. Triethylamine (1.29 mL, 9.22 mmol, 2.2 equiv.) was added; a condenser was used to avoid evaporative loss of the base catalyst. 10-Undecenoyl chloride (1.70 g, 8.36 mmol, 2.0 equiv.) was added dropwise and allowed to react at 90°C for 20 h. The reaction mixture was then filtered, and the filtrate was precipitated in 300 mL 50:50 water/ethyl alcohol. The precipitate was redissolved in a minimal amount of CH_2Cl_2 and reprecipitated in hexane. The product was washed with hexane and dried under vacuum at 40°C.

^{1}H NMR (DMSO-d_6): 1.21 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2CH=CH_2$), 1.32 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2CH=CH_2$), 1.50 (br s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$), 1.8-2.1(m, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$, and $COC\underline{H}_3$), 2.30 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CH_2$), 3.3-5.3 (m, cellulose backbone), 4.8-5.0 (q, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}_2$), 5.7 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CH_2$). ^{13}C NMR ($CDCl_3$): 20.4 ($CO\underline{C}H_3$), 24.8 ($COCH_2\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$), 28.8 ($COCH_2CH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2CH_2CH=CH_2$), 33.6 ($CO\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2\underline{C}H_2CH=CH_2$), 114.1 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=\underline{C}H_2$), 139.0 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2\underline{C}H=CH_2$), 168.9-173.1 (C=O), 62.2 (C-6), 72.0-76.4 (C2,

C3, C5), 82.3 (C-4), 100.7 (C-1). Degree of substitution (DS) by ^{1}H NMR: DS(10-undecenoate) (DS(Un)) 0.67, DS(acetate) (DS(Ac)) 1.88; yield: 93%.

Preparation of cellulose acetate 4-pentenoate (CA-Pen, 3). CA-320S (**1**, 1.00 g, 4.19 mmol/AGU) was dissolved in DMI (20 mL), and the solution was heated to 90°C with mechanical stirring under N_2. Triethylamine (2.6 mL, 9.22 mmol, 2.2 equiv.) was added; a condenser was used to avoid evaporative loss of the base catalyst. 4-Pentenoyl chloride (1.99 g, 8.38 mmol, 2.0 equiv.) was added dropwise and allowed to react at 90°C for 20 h. The reaction mixture was then filtered, and the filtrate was precipitated in 300 mL 50:50 water/ethyl alcohol. The precipitate was redissolved in a minimal amount of CH_2Cl_2 and reprecipitated in hexane. The product was washed with hexane and dried under vacuum at 40°C.

^{1}H NMR (DMSO-d_6): 1.8-2.1(m, COC$\underline{H}_3$), 2.28 (br s, COCH$_2$C$\underline{H}_2$CH=CH$_2$), 2.42 (br s, COC$\underline{H}_2$CH$_2$CH=CH$_2$), 2.9-5.3 (m, cellulose backbone), 4.9-5.1 (q, COCH$_2$CH$_2$CH=C$\underline{H}_2$), 5.8 (m, COCH$_2$CH$_2$C$\underline{H}$=CH$_2$). ^{13}C NMR (DMSO-d_6): 20.4 (CO$\underline{C}$H$_3$), 28.6 (COCH$_2$$\underline{C}H_2$CH=CH$_2$), 32.8 (CO$\underline{C}H_2CH_2$CH=CH$_2$), 116.0 (COCH$_2CH_2$CH=$\underline{C}H_2$), 137.3 (COCH$_2CH_2$$\underline{C}$H=CH$_2$), 168.9-173.1 (C=O), 62.2 (C-6), 72.0-76.4 (C2, C3, C5), 82.3 (C-4), 100.7 (C-1). Degree of substitution (DS) by ^{1}H NMR: DS(4-pentenoate) (DS(Pen)) 0.56, DS(acetate) (DS(Ac)) 1.80; yield: 89%.

5.2.4 Olefin cross-metathesis

General procedure for olefin cross-metathesis reactions. To a flask charged with cellulose derivative **2** CA-Un or **3** CA-Pen (100 mg, 1.0 equiv. olefin), 5 mg BHT and 5 mL anhydrous THF were added. After the reagents were completely dissolved, cross-metathesis partner (acrylic acid, methyl acrylate, 2-hydroxylethyl acrylate, poly(ethylene glycol) methyl ether acrylate, allyl amine, or allyl alcohol; 20 equiv.) was added followed by the addition of Hoveyda-Grubbs Catalyst 2nd Generation (0.05 equiv. in 2 mL THF) via syringe. After stirring for 1h under N_2 at 40°C, the reaction was stopped by adding 1-2 drops of diethylene glycol monovinyl ether or ethyl vinyl ether. The product was collected by either dialysis and freeze-drying, or by precipitating in H_2O/ethanol followed by sufficient washing by H_2O before being dried under vacuum at 40°C.

2a. Cellulose acetate 10-undecenoate (2) CM with acrylic acid

^{1}H NMR (DMSO-d_6): 1.23 (br s, COCH$_2$CH$_2$C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$CH$_2$CH$_2$CH=CHCOOH), 1.38 (br s, COCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$C$\underline{H}_2$CH$_2$CH=CHCOOH), 1.50 (br. s,

$COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOH$), 1.86-2.05(m, $COC\underline{H}_3$), 2.14 (br s, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOH$), 2.28 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CHCOOH$), 2.75-5.25 (m, cellulose backbone), 5.68(d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOH$, *Z* configuration), 5.74 (d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOH$, *E* configuration), 6.19 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOH$, *Z* configuration), 6.80 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOH$, *E* configuration). ^{13}C NMR (DMSO-d_6): 20.7 ($CO\underline{C}H_3$), 24.8 ($COCH_2\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOH$), 28.0 ($COCH_2CH_2CH_2CH_2CH_2CH_2\underline{C}H_2CH_2CH=CHCOOH$), 29.0 ($COCH_2CH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2CH_2CH_2CH=CHCOOH$), 31.8 ($CO\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOH$), 33.8 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2\underline{C}H_2CH=CHCOOH$), 122.4 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=\underline{C}HCOOH$), 149.0 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=\underline{C}HCOOH$), 167.4 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH\underline{C}OOH$), 169.1-173.3 (C=O), 63.0 (C-6), 72.0-76.4 (C2, C3, C5), 80.4 (C-4), 100.0 (C-1). Conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: 16.7, yield: 93%.

2b. Cellulose acetate 10-undecenoate (2) CM with 2-hydroxyethyl acrylate

^{1}H NMR (DMSO-d_6): 1.22 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2CH=CHCOOCH_2CH_2OH$), 1.37 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2CH=CHCOOCH_2CH_2OH$), 1.50 (br. s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOCH_2CH_2OH$), 1.8-2.1 (m, $COC\underline{H}_3$), 2.18 (br s, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOCH_2CH_2OH$), 2.28 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CHCOOCH_2CH_2OH$), 2.75-5.25 (m, cellulose backbone), 3.56 (t, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOCH_2C\underline{H}_2OH$), 4.06 (t, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOC\underline{H}_2CH_2OH$), 5.77(d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOCH_2CH_2OH$, *Z* configuration), 5.86 (d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOCH_2CH_2OH$, *E* configuration), 6.28 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOCH_2CH_2OH$, *Z* configuration), 6.89 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOCH_2CH_2OH$, *E* configuration). ^{13}C NMR (DMSO-d_6): 20.9 ($CO\underline{C}H_3$), 24.8

($COCH_2\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOCH_2CH_2OH$), 27.9
($COCH_2CH_2CH_2CH_2CH_2CH_2\underline{C}H_2CH_2CH=CHCOOCH_2CH_2OH$), 29.0
($COCH_2CH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2CH_2CH_2CH=CHCOOCH_2CH_2OH$), 31.8
($CO\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOCH_2CH_2OH$), 33.9
($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2\underline{C}H_2CH=CHCOOCH_2CH_2OH$), 59.6
($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOCH_2\underline{C}H_2OH$), 65.9
($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOO\underline{C}H_2CH_2OH$), 121.4
($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=\underline{C}HCOOCH_2CH_2OH$), 149.8
($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=\underline{C}HCOOCH_2CH_2OH$), 166.2
($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH\underline{C}OOCH_2CH_2OH$), 169.1-173.3 (C=O), 63.0 (C-6), 72.0-76.4 (C2, C3, C5), 80.4 (C-4), 100.0 (C-1). Conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: 15.2, yield: 97%.

2c. Cellulose acetate 10-undecenoate (2) CM with poly(ethyl glycol) methyl ether acrylate

^{1}H NMR (DMSO-d_6): 1.25 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2CH=CHCOO(CH_2CH_2O)_xCH_3$), 1.41 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2CH=CHCOO(CH_2CH_2O)_xCH_3$), 1.49 (br. s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOO(CH_2CH_2O)_xCH_3$), 1.8-2.1(m, $COC\underline{H}_3$), 2.18 (br s, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOO(CH_2CH_2O)_xH$), 2.28 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CHCOO(CH_2CH_2O)_xCH_3$), 2.75-5.25 (m, cellulose backbone), 3.23(s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOO(CH_2CH_2O)_xC\underline{H}_3$), 3.42, 3.50, 3.61 and 4.17 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOO(C\underline{H}_2C\underline{H}_2O)_xCH_3$), 5.77 (d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COO(CH_2CH_2O)_xCH_3$, *Z* configuration), 5.86 (d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COO(CH_2CH_2O)_xCH_3$, *E* configuration), 6.28 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOO(CH_2CH_2O)_xCH_3$, *Z* configuration), 6.89 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOO(CH_2CH_2O)_xCH_3$, *E* configuration). Conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: 33.3, yield: 84%.

2d. Cellulose acetate 10-undecenoate (2) CM with methyl acrylate

^{1}H NMR (DMSO-d_6): 1.24 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2CH=CHCOOCH_3$), 1.39 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2CH=CHCOOCH_3$), 1.50 (br. s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOCH_3$), 1.8-2.1(m, $COC\underline{H}_3$), 2.17 (br s, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCOOCH_3$), 2.28 (br s,

$COCH_2CH_2CH_2CH_2CH_2CH_2CH_2\underline{H}_2CH=CHCOOCH_3$), 2.75-5.25 (m, cellulose backbone), 5.77 (d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOCH_3$, *Z* configuration), 5.83 (d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}COOCH_3$, *E* configuration), 6.27 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOCH_3$, *Z* configuration), 6.87 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CHCOOCH_3$, *E* configuration). Conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: 16.7, yield: 94%.

3a. Cellulose acetate 4-pentenoate CM with acrylic acid

^{1}H NMR (DMSO-d_6): 1.8-2.1(m, $COC\underline{H}_3$), 2.43 (br s, $COC\underline{H}_2C\underline{H}_2CH=CHCOOH$), 2.9-5.3 (m, cellulose backbone), 5.78 (m, $COCH_2CH_2CH=C\underline{H}COOH$, *E* and *Z* configuration), 6.19 (m, $COCH_2CH_2C\underline{H}=CHCOOH$, *Z* configuration), 6.79 (m, $COCH_2CH_2CH=C\underline{H}COOH$, *E* configuration). Conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: 15.5, yield: 94%.

3b. Cellulose acetate 4-pentenoate CM with 2-hydroxyethyl acrylate

^{1}H NMR (DMSO-d_6): 1.8-2.1(m, $COC\underline{H}_3$), 2.45 (br s, $COC\underline{H}_2C\underline{H}_2CH=CHCOOCH_2CH_2OH$), 2.9-5.3 (m, cellulose backbone), 3.60 (t, $COCH_2CH_2CH=CHCOOCH_2C\underline{H}_2OH$), 4.07 (t, $COCH_2CH_2CH=CHCOOC\underline{H}_2CH_2OH$), 5.88 (m, $COCH_2CH_2CH=C\underline{H}COOCH_2CH_2OH$, *E* and *Z* configuration), 6.28 (m, $COCH_2CH_2C\underline{H}=CHCOOCH_2CH_2OH$, *Z* configuration), 6.90 (m, $COCH_2CH_2CH=C\underline{H}COOH$, *E* configuration). ^{13}C NMR (DMSO-d_6): 20.6 ($CO\underline{C}H_3$), 27.9 ($COCH_2\underline{C}H_2CH=CHCOOCH_2CH_2OH$), 30.9 ($CO\underline{C}H_2CH_2CH=CHCOOCH_2CH_2OH$), 59.4 ($COCH_2CH_2CH=CHCOOCH_2\underline{C}H_2OH$), 66.1 ($COCH_2CH_2CH=CHCOO\underline{C}H_2CH_2OH$), 121.8 ($COCH_2CH_2CH=\underline{C}HCOOCH_2CH_2OH$), 148.0 ($COCH_2CH_2CH=\underline{C}HCOOCH_2CH_2OH$), 166.1 ($COCH_2CH_2CH=CH\underline{C}OOCH_2CH_2OH$), 169.1-173.3 (C=O), 63.0 (C-6), 72.0-76.4 (C2, C3, C5), 80.4 (C-4), 100.0 (C-1). Conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: 9.7, yield: 96%.

3c. Cellulose acetate 4-pentenoate CM with poly(ethyl glycol) methyl ether acrylate

^{1}H NMR (DMSO-d_6): 1.8-2.1(m, $COC\underline{H}_3$), 2.44 (br s, $COC\underline{H}_2C\underline{H}_2CH=CHCOO\ (CH_2CH_2O)_x\ CH_3$), 2.75-5.25 (m, cellulose backbone), 3.23(s, $COCH_2CH_2CH=CHCOO\ (CH_2CH_2O)_x\ C\underline{H}_3$), 3.41, 3.50, 3.61 and 4.17 (m, $COCH_2CH_2CH=CHCOO\ (C\underline{H}_2C\underline{H}_2O)_x\ CH_3$), 5.90(m, $COCH_2CH_2CH=C\underline{H}COO\ CH_3$, *E* and *Z* configuration), 6.31 (m, $COCH_2CH_2C\underline{H}=CHCOO\ CH_3$, *Z* configuration), 6.90 (m, $COCH_2CH_2C\underline{H}=CHCOO\ CH_3$, *E* configuration). Conversion by ^{1}H NMR: 100%, *E/Z* ratio by ^{1}H NMR: 8.3, yield: 88%.

5.2.5 Hydrogenation

General procedure for reduction of the α.β-unsaturated double bond of the CM products by Pd/C hydrogenation. To a solution of 500 mg CM product dissolved in 50 mL anhydrous THF, 150 mg palladium on carbon (10 wt% loading) was added. The mixture was stirred overnight under 80 psi H_2 at room temperature (for compound **3** based sample **3a**, after filtering the mixture through Celite, another 150 mg Pd/C was added and reacted under 80 psi H_2 for 12 hours. The cycle was repeated once more (total of three hydrogenations) to make sure that all the double bonds were hydrogenated). The mixture was filtered through Celite, concentrated, and then precipitated into hexanes. The precipitate was collected and dried under vacuum at 40°C.

General procedure for olefin cross-metathesis/hydrogenation one-pot reaction. To a Parr Reactor (vessel volume: 600 mL) charged with cellulose derivative **2** or **3** (400 mg, 1.0 equiv. olefin), 20 mg BHT and 40 mL anhydrous THF were added. After the reagents were completely dissolved, the cross-metathesis partner (acrylic acid, methyl acrylate, 2-hydroxylethyl acrylate, poly(ethylene glycol) methyl ether acrylate, or allyl alcohol; 20 equiv.) was added followed by the addition of Hoveyda-Grubbs Catalyst 2nd Generation (0.05 equiv. in 6 mL THF) via syringe. After stirring for 1 hour under N_2 at room temperature, 30 wt % Pd/C was added. The mixture was stirred under 80 psi H_2 at room temperature. The subsequent reaction and purification followed that in the general procedure above.

2a'. Hydrogenation product of 2a

^{1}H NMR (DMSO-d_6): 1.23 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2COOH$), 1.47 (br s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CHC\underline{H}_2CH_2COOH$), 1.8-2.1(m, $COC\underline{H}_3$), 2.17 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2COOH$), 2.28 ($COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$), 2.75-5.25 (m, cellulose backbone). Yield: 90%.

2b'. Hydrogenation product of 2b

^{1}H NMR (DMSO-d_6): 1.24 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2COOCH_2CH_2OH$), 1.51 (br. s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2COOCH_2CH_2OH$), 1.8-2.1(m, $COC\underline{H}_3$), 2.2-2.4 (m, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2COOCH_2CH_2OH$), 2.75-5.25 (m, cellulose backbone), 3.54 (t, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOCH_2C\underline{H}_2OH$), 4.00 (t, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOC\underline{H}_2CH_2OH$). ^{13}C NMR (DMSO-$d_6$): 20.9

($CO\underline{C}H_3$), 24.9 ($COCH_2\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOCH_2CH_2OH$), 28.9 ($COCH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2CH_2COOCH_2CH_2OH$), 33.9 ($CO\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2\underline{C}H_2COOCH_2CH_2OH$), 59.4 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOCH_2\underline{C}H_2OH$), 65.9 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COO\underline{C}H_2CH_2OH$), 173.4 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2\underline{C}OOCH_2CH_2OH$), 169.1-173.3 (C=O), 63.0 (C-6), 72.0-76.4 (C2, C3, C5), 80.4 (C-4), 100.0 (C-1).Yield: 93%.

2c'. Hydrogenation product of 2c

^{1}H NMR (DMSO-d_6): 1.23 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2COO(CH_2CH_2O)_xCH_3$), 1.50 (br s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2COO(CH_2CH_2O)_xCH_3$), 1.8-2.1(m, $COC\underline{H}_3$), 2.27 (m, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2COO(CH_2CH_2O)_xCH_3$), 2.75-5.25 (m, cellulose backbone), 3.23(s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COO(CH_2CH_2O)_xC\underline{H}_3$), 3.42, 3.49, 3.58 and 4.10 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COO(C\underline{H}_2C\underline{H}_2O)_xCH_3$). Yield: 79%.

2d'. Hydrogenation product of 2d

^{1}H NMR ($CDCl_3$): 1.28 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2COOCH_3$), 1.61 (br s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2COOCH_3$), 1.9-2.1 (m, $COC\underline{H}_3$), 2.28 (m, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2COO(CH_2CH_2O)_xCH_3$), 3.23-5.25 (m, cellulose backbone), 3.66 (s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOC\underline{H}_3$). Yield: 89%.

3a'. Hydrogenation product of 3a

^{1}H NMR (DMSO-d_6): 0.81and 1.25 (alkyl CH_2 and CH_3), 1.8-2.1(m, $COC\underline{H}_3$), 2.49 ($COCH_2C\underline{H}_2C\underline{H}_2CH_2COOH$), 2.16 and 2.30 ($COC\underline{H}_2CH_2CH_2C\underline{H}_2COOH$), 2.9-5.3 (m, cellulose backbone). Yield: 79%.

3b'. Hydrogenation product of 3b

^{1}H NMR (DMSO-d_6): 0.86 and 1.28 (alkyl CH_2 and CH_3), 1.54 (br s, $COCH_2C\underline{H}_2C\underline{H}_2CH_2COOCH_2CH_2OH$), 1.8-2.1(m, $COC\underline{H}_3$), 2.30 (br s, $COC\underline{H}_2CH_2CH_2C\underline{H}_2COOCH_2CH_2OH$), 2.9-5.3 (m, cellulose backbone), 3.56 (t, $COCH_2CH_2CH_2CH_2COOCH_2C\underline{H}_2OH$), 4.02 (t, $COCH_2CH_2CH_2CH_2COOC\underline{H}_2CH_2OH$). ^{13}C NMR (DMSO-$d_6$): 20.5 ($CO\underline{C}H_3$), 24.2 ($COCH_2\underline{C}H_2\underline{C}H_2CH_2COOCH_2CH_2OH$), 33.5 ($CO\underline{C}H_2CH_2CH_2\underline{C}H_2COOCH_2CH_2OH$), 30.9 ($CO\underline{C}H_2CH_2CH{=}CHCOOCH_2CH_2OH$), 59.4

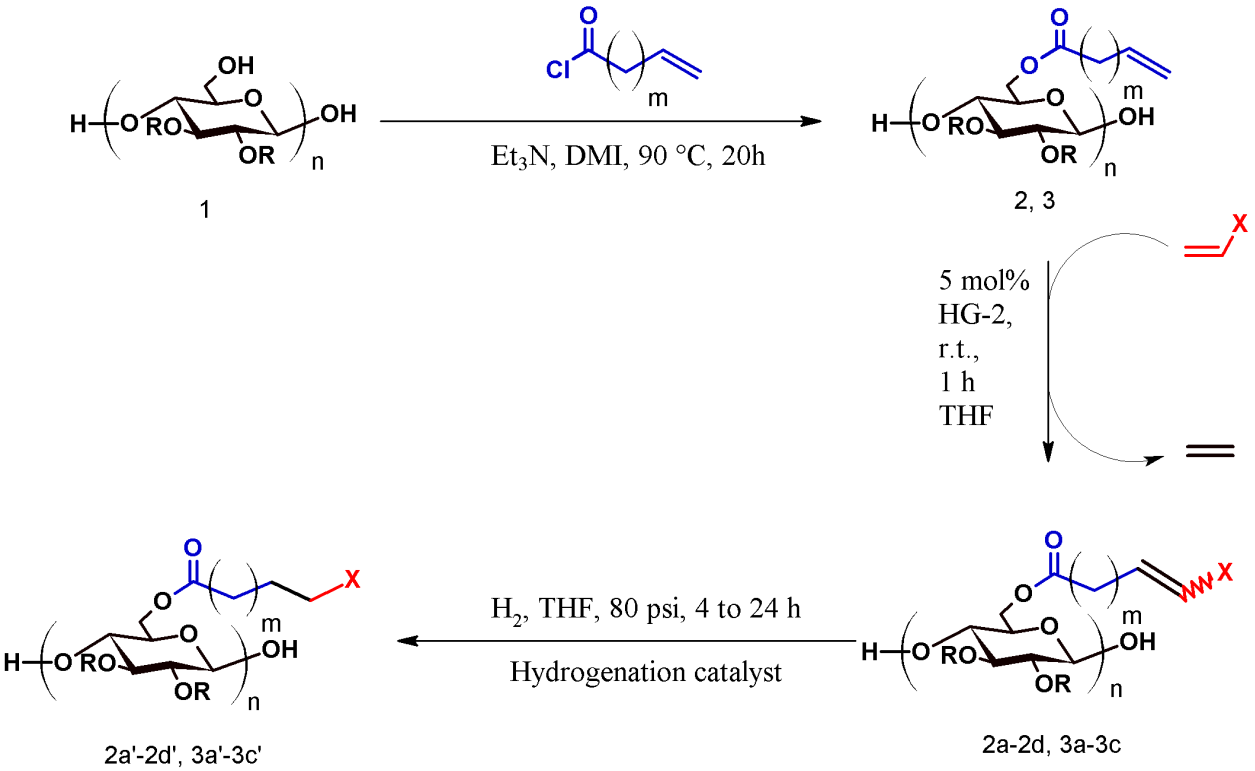
($COCH_2CH_2CH_2CH_2COOCH_2\underline{C}H_2OH$), 66.0 ($COCH_2CH_2CH_2CH_2COO\underline{C}H_2CH_2OH$), 173.2($COCH_2CH_2CH_2CH_2\underline{C}OOCH_2CH_2OH$), 169.1-173.3 (C=O), 63.0 (C-6), 72.0-76.4 (C2, C3, C5), 80.4 (C-4), 100.0 (C-1). Yield: 74%.

3c'. Hydrogenation product of 3c

0.86 and 1.28 (alkyl CH_2 and CH_3), 1.54 (br s, $COCH_2C\underline{H}_2C\underline{H}_2CH_2COO(CH_2CH_2O)_xCH_3$), 1.8-2.1(m, $COC\underline{H}_3$), 2.30 (br s, $COC\underline{H}_2CH_2CH_2C\underline{H}_2COO(CH_2CH_2O)_xCH_3$), 2.9-5.3 (m, cellulose backbone), 3.23(s, $COCH_2CH_2CH_2CH_2COO\ (CH_2CH_2O)_x\ C\underline{H}_3$), 3.41, 3.51, 3.58 and 4.12 (m, $COCH_2CH_2CH_2CH_2COO(C\underline{H}_2C\underline{H}_2O)_x\ CH_3$). Yield: 67%.

5.3 Results and discussion

Our previous results[34] showed the power of olefin CM for synthesis of carboxylic acid functionalized cellulose derivatives. Using the selective Hoveyda-Grubbs' 2nd generation catalyst, cellulose alkanoate undec-10-enoate esters (e.g., cellulose acetate propionate undec-10-enoate) were reacted with acrylic acid, resulting in full conversion to cellulose ω-carboxyalkanoates within 1 hour at mild temperatures (40 ºC). Not only did this approach provide a new pathway to a broader variety of cellulose ω-carboxyalkanoates, but also raised the possibility that this might be a more widely useful synthetic approach.

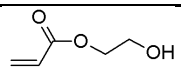
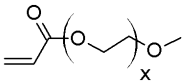
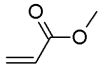
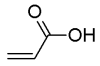
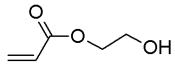
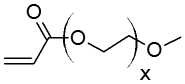


[a]Note that structures are not meant to imply regiospecificity; particular positions of substitution in all schemes are only for convenience of depiction and clarity.

Figure 5.3. General three-step synthetic method for functionalized cellulose esters via olefin cross-metathesis[a]

Table 5.1. CM of olefin terminated cellulose acetate with acrylic acid and acrylates.

Cpd.	Starting cellulose ester	CM partner (abbr.)	E/Z ratio[a]	Conversion[a]	Yield %	DS (X)[a]	DS (Ac)[a]	Hydrogenation Product
1	-	-	-	-	-	-	1.82[b]	-
2	1	-	-	-	93	0.67	1.88	-
2a	2	 (AA)	16.7	~100%	93	0.77	1.77	**2a'**

2b	2	 (HEA)	15.2	~100%	97	0.65	1.78	**2b'**
2c	2	 (PEGMEA)	33.3	~100%	84	NA	NA	**2c'**
2d	2	 (MA)	16.7	~100%	94	0.67	NA	**2d'**
3	1	-	-	-	89	0.56	1.80	-
3a	3	 (AA)	15.5	~100%	94	0.56	1.85	**3a'**
3b	3	 (HEA)	9.7	~100%	96	0.56	1.86	**3b'**
3c	3	 (PEGMEA)	8.3	~100%	88	NA	NA	**3c'**

[a]Determined by proton NMR; [b]Reported by supplier.

Cellulose acetate undec-10-enoate with DS 0.67 of undec-10-enoate (**2**, m = 8 in **Figure 5.3**) was first chosen as starting material. Eight methylene groups separate the terminal olefin and ester carbonyl, providing high olefin electron density and minimal steric hindrance, and making this terminal olefin a reactive substrate for Grubbs' catalysts[33]. We first tested the efficiency of allyl alcohol as a CM partner, but observed only about 60% conversion. Replacing allyl alcohol with 3-buten-2-ol, which has a more sterically hindered olefin and would hence be less prone to self-metathesis, did not improve conversion (data not shown). According to Grubbs' CM rules[33] reaction efficiency depends largely on catalyst type, as well substrate reactivity. Generally, type I olefins (electron rich and/or less sterically hindered, e.g. terminal olefins) are reactive but unselective. In contrast, electron deficient and/or sterically hindered olefins, which can be categorized into type II or III olefins (e.g. acrylic acid and acrylates) are less reactive but more selective. While an olefin metathesis reaction between a type I olefin and a type II or type III olefin tends to give CM products, a reaction between two type I olefins is more

likely to generate a mixture of CM and self-metathesis (SM) products. Apparently the olefin electron density of allyl alcohols is sufficient to make them react as type I olefins, leading to competition with allyl alcohol homodimerization and suboptimal conversions[40]. Note however that in some cases 60% conversion could be more than adequate, especially since residual double bonds can be readily reduced (*vide infra*).

Unprotected allyl amine on the other hand provided no evidence of conversion to CM products (data not shown). This is likely due not only to the low selectivity of the electron-rich double bond of allyl amine, but also to the likely coordination of the amine with ruthenium, thereby suppressing its catalytic activity[41,42].

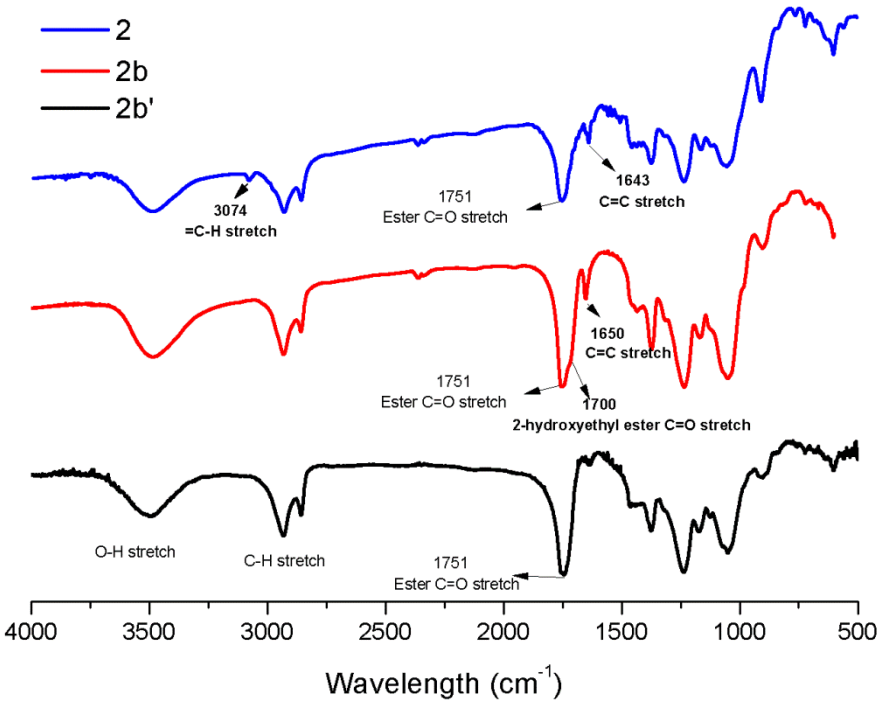


Figure 5.4. FTIR spectra of terminally olefinic cellulose acetate undec-10-enoate 2, the CM product (with HEA) 2b, and the hydrogenated product 2b'.

These unsatisfying results led us to acrylates, which are typically type II or III olefins due to the proximity of the electron-withdrawing carbonyl group to the olefin. In this work we studied three representative acrylate partners; the simple ester methyl acrylate (MA), as well as two acrylate esters with bifunctional alcohols that could be used to append other functionality, namely 2-hydroxyethyl acrylate (HEA) and poly(ethylene glycol) methyl ether acrylate (PEGMEA) (average M_n= 480 Da). We included acrylic acid (AA) for comparison, especially

for the cellulose pentenoate examples (Table 1). We were pleased to find that all examined acrylates and acrylate esters gave nearly 100% conversion with perfect selectivity for CM products under mild conditions (40°C, 1h, THF, 5 mol % Hoveyda-Grubbs catalyst, 20:1 ratio of acrylate to cellulose ester). CM reaction with HEA will serve to exemplify the results obtained; characterization data for products obtained by reaction with other CM partners can be found in the **Supporting Information**. Successful CM was supported by the FTIR spectra (**Figure 5.4**). For example, the peak at 3074 cm^{-1} in the spectrum of starting cellulose undec-10-enoate **2**, assigned to CHR stretch of the terminal olefin $RCH=CH_2$, is absent from the spectrum of the new CM product **2b**. A new absorbance at 1700 cm^{-1}, assigned to the C=O stretch of the α,β-unsaturated 2-hydroxyethyl ester, was observed for **2b** on the shoulder of the cellulose ester C=O stretch peak at 1751 cm^{-1}. Further, the C=C stretch signal of the terminal olefin ($CHR=CH_2$) in **2** at 1643 cm^{-1} was shifted to higher frequency 1650 cm^{-1} (CHR=CHR (mostly trans as determined by ^1H NMR)).

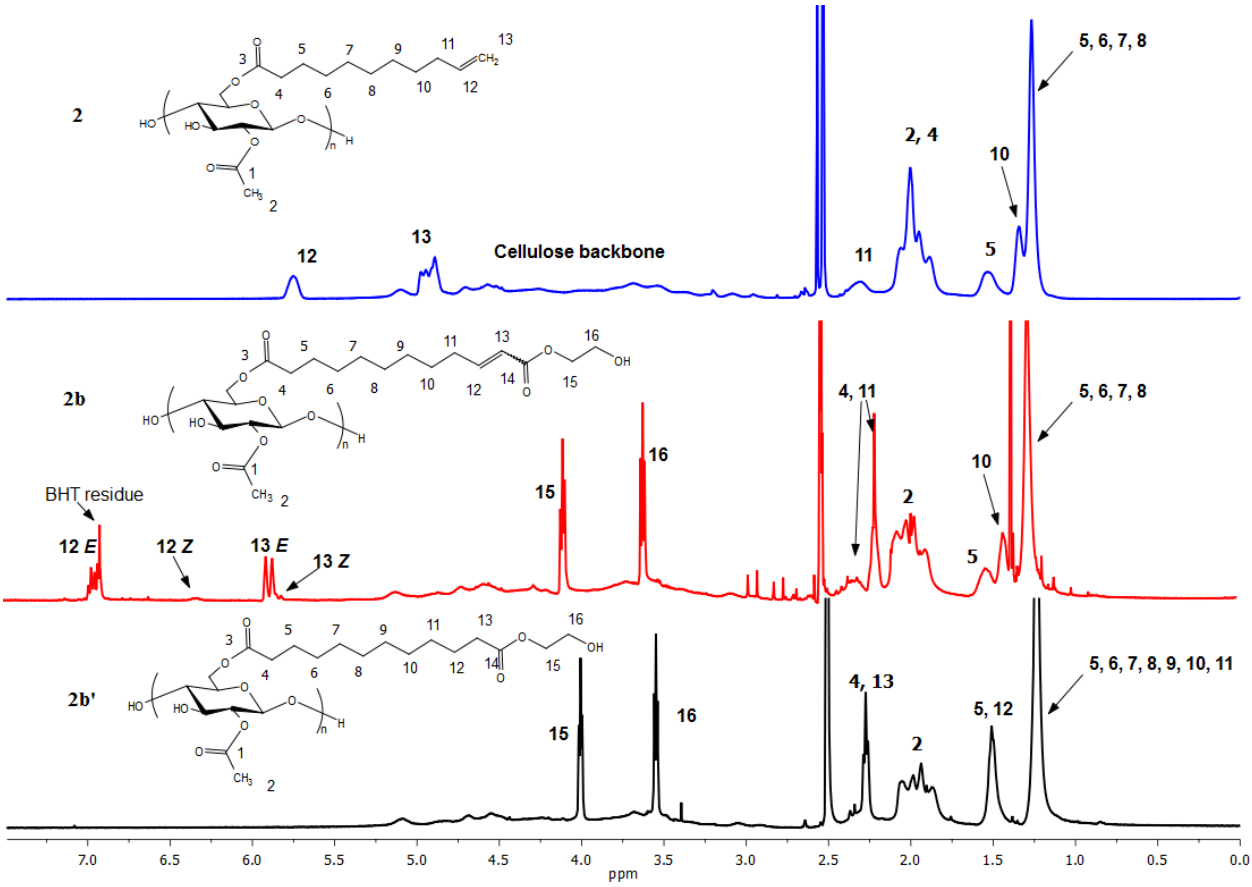


Figure 5.5. ^1H NMR spectra of terminally olefinic cellulose acetate undec-10-enoate 2, CM product (with HEA) 2b, and hydrogenated product 2b'.

Proton NMR (**Figure 5.5**) is an excellent tool for monitoring the CM reaction; disappearance of the terminal olefin signals of **2** at 4.90 and 5.75 ppm, and emergence of new downfield signals for the α,β-unsaturation (*E* configuration, 5.86 and 6.89 ppm for **2b**), are diagnostic for occurrence and extent of CM, since these resonances are sharp and well-separated from each other and all other resonances of these cellulose derivatives. The corresponding signals of olefinic protons from the minor *Z* configuration product were observed at 5.77 and 6.28 ppm (*Z/E* double bond ratios determined by integration of these signals are listed in Table 1). Complete conversion of terminal olefin to α,β-unsaturated ester was thereby confirmed. We did not expect that there would be loss of the acyl groups (e.g. acetate) under these mild reaction conditions, but the ^{1}H NMR spectra allowed us to affirm this hypothesis. Substituent DS was calculated by ^{1}H NMR integration (Table 1), confirming that under these benign CM conditions the ester substituents largely remain untouched. ^{13}C NMR analysis provides further evidence of the clean and complete nature of these CM reactions (**Figure 5.6**). The terminal olefin resonances of compound **2** at 114 and 139 ppm completely disappeared, while new peaks that were assigned to the α, β-unsaturated carbons of **2b** appeared downfield at 122 and 148 ppm.

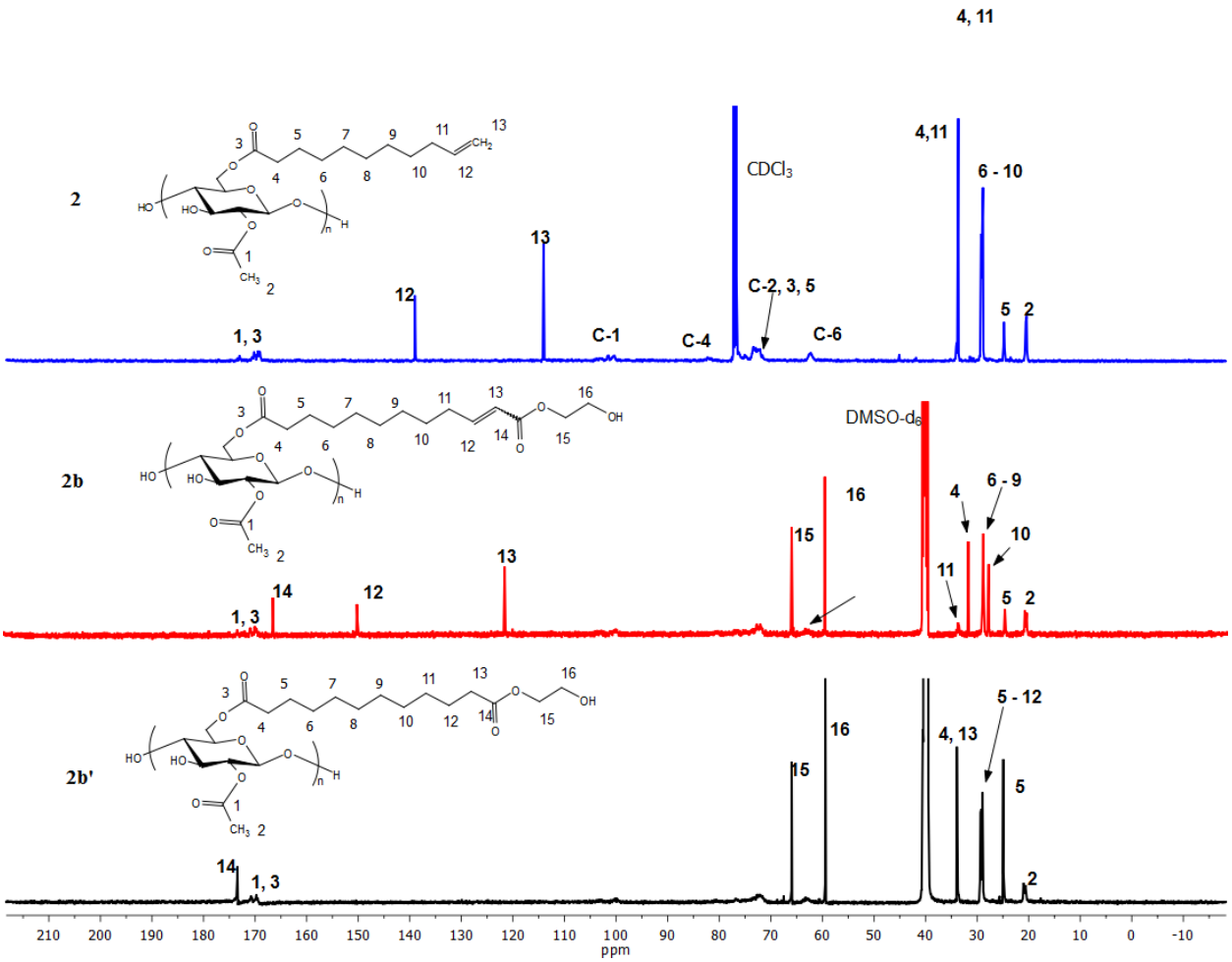


Figure 5.6. ^{13}C NMR of of terminally olefinic cellulose acetate undec-10-enoate 2, CM product (with HEA) 2b, and hydrogenated product 2b'.

Similar results (Supporting Information) were observed in CM reactions between **2** and AA, PEGMEA (average M_n= 480 Da), and MA, giving complete conversion to CM products **2a**, **2c** and **2d** respectively. Although it was not surprising that small acrylates like HEA and MA were effective CM partners, we were pleasantly surprised to observe complete CM conversion with a PEG functionalized acrylate, PEGMEA, considering the potential for steric interference between the cellulose chain and that of the polymeric PEGMEA (average M_n of 480 Da). This invites speculation that a variety of acrylate end-functionalized polymers might also be feasible CM partners for polysaccharide derivatives like these. This modular reaction, like click reactions such as the azide-alkyne Huisgen cycloaddition reaction[25,27,43] and "thiol-ene" reaction[28,29,44], may create potential for the synthesis of polysaccharide-based graft copolymers by a "grafting to" approach.

In our previous work[34], we noticed that the CM products of cellulose 10-undecenoate esters and acrylic acid appeared to crosslink during storage. We proposed that free radical oligomerization of the pendant α,β-unsaturated carboxylic acids was responsible for the crosslinking, as supported by the fact that added free radical scavenger (BHT) suppressed the crosslinking. In the current study, similar phenomena were observed for some of our products. Although adding BHT delayed the crosslinking process, the CM products became insoluble after weeks or months of storage, which may be attributed to consumption of the free radical scavenger, and/or the involvement of other crosslinking mechanisms (e.g. secondary olefin metathesis due to residual catalyst, although we consider this unlikely).

Considering that most imaginable crosslinking mechanisms are related to the α,β-unsaturation, it should be possible to eliminate the possibility of crosslinking if one is able to reduce the olefin. Palladium catalyzed hydrogenation has been previously used in carbohydrate and polysaccharide chemistry to reduce double bonds[45] without impacting other functional groups. Therefore we pursued palladium-catalyzed reduction of CM product olefins. Purified, dried CM products were hydrogenated (H_2, Pd/C (10 wt % Pd (dry basis)/C)) at room temperature in THF. For the undec-10-enoate-based derivatives (**2a-2d**), only ca. 50% olefin hydrogenation was observed using a hydrogen balloon and Pd/C. Higher hydrogen pressure (80 psi) in a Parr reactor was more successful, affording fully hydrogenated products (**2a'-2d'**) (**Table 5.2**). ^{1}H NMR spectra of **2b** and **2b'** (**Figure 5.5** and **S7-9**) showed that both *E* olefinic protons at 5.8 and 6.9 ppm and *Z* olefinic protons at 5.3 and 6.7 ppm were entirely absent from the spectrum of **2b'**, indicating complete olefin hydrogenation. The ^{13}C NMR spectrum of 2b provided further confirmation, showing disappearance of the α,β-unsaturated double bond carbon signals at 122 and 148 ppm in **2b** after hydrogenation. FTIR spectra of **2b'** (**Figure 5.4** and **S1-3**), show disappearance of the previous shoulder peak at 1700 cm^{-1}, which was assigned to the C=O stretch of the α,β-unsaturated methyl ester **2b**. Moreover, after hydrogenation, the sharp C=C stretch (CHR=CHR, *trans*) signal around 1650 cm^{-1} also disappeared, revealing the previously hidden H_2O vibrational peak.

Since THF is a good solvent for both the CM reaction and the subsequent hydrogenation, we wished to explore the potential efficiency of a one-pot reaction. Therefore, hydrogenation catalyst (Pd/C) was added directly to the reaction mixture after completion of CM, and the reaction mixture subjected to hydrogen pressure (80 psi). After hydrogenation, product ^{1}H NMR

and FTIR spectra were identical to those of products obtained by the two-step pathway, showing the feasibility of this one-pot synthesis. Considering the possibility of crosslinking (sometimes rapid) of olefin-containing CM products already described (*vide supra*), the potential for immediate, one-pot olefin reduction is of special importance.

We expected that the length of the tether between the terminal double bond and the ester carbonyl might impact CM efficiency, due to the potential for steric interference of the cellulose main chain with ruthenium complexation in the cases of shorter tethers. To test the potential for such effects, cellulose acetate pent-4-enoate (DS pent-4-enoate 0.56, (CA-Pen, **3**)) was synthesized and used in CM reactions. Reaction of CM partners including AA, HEA and PEGMEA with CA-Pen under the same conditions as used for the reactions with the undec-10-enoate esters showed similar results. Full conversion to CM products was achieved (^{1}H NMR, FTIR, **S4-6**, **S10-11**), with essentially no loss of ester substituents during CM. These results show that a 2-carbon spacer between ester carbonyl and terminal double bond is enough for successful, complete CM reaction.

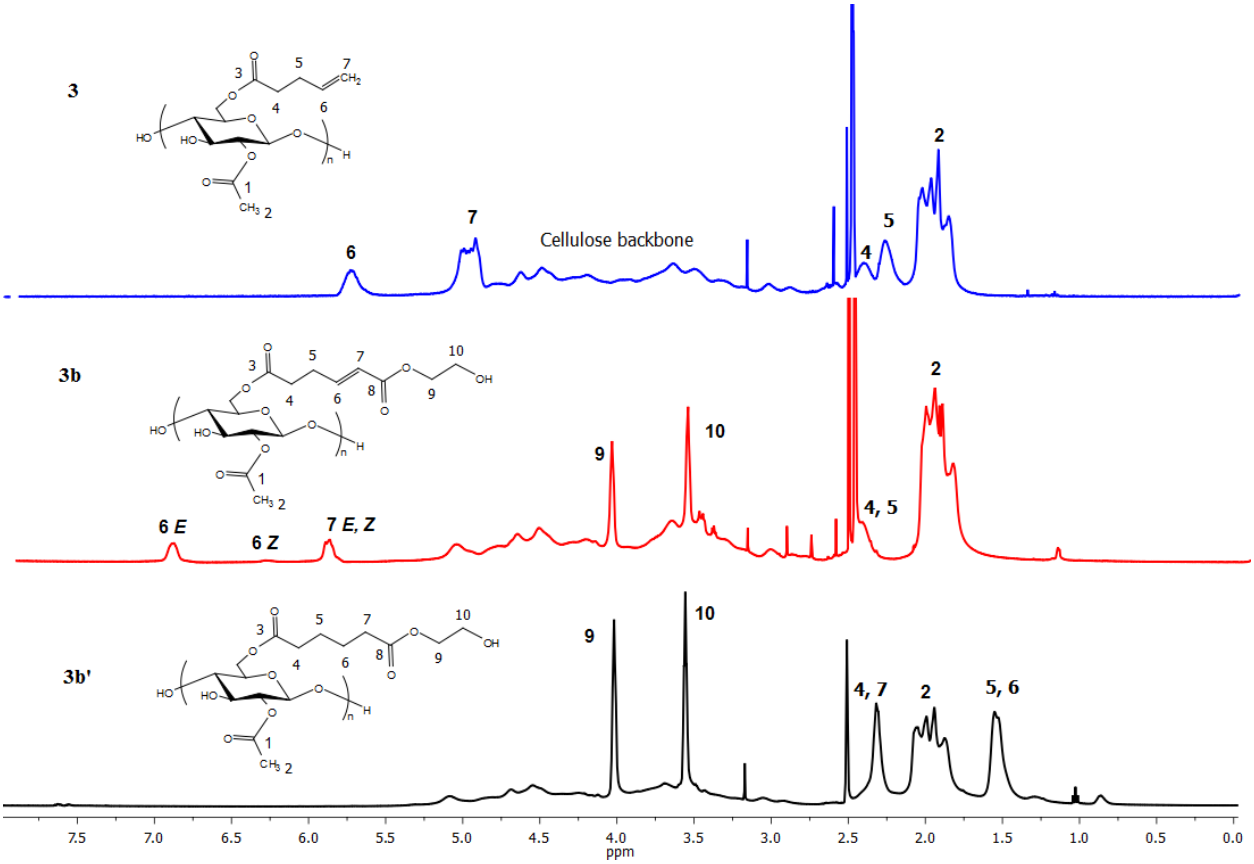


Figure 5.7. [1]H NMR spectra of terminally olefinic cellulose acetate pent-4-enoate 3, CM product (with HEA) 3b, and hydrogenated product 3b'.

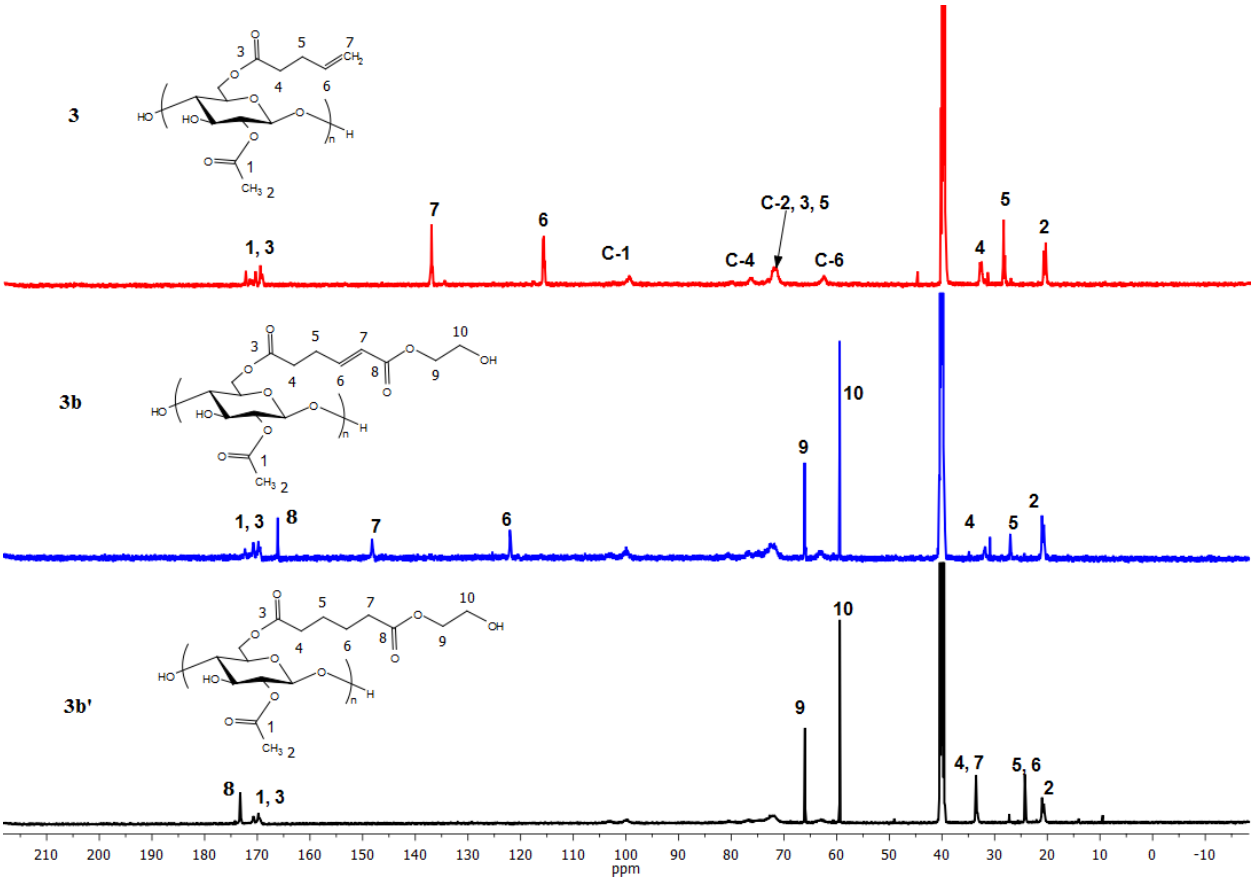
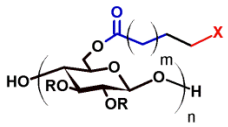


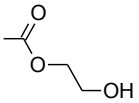
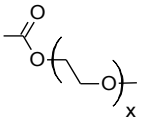
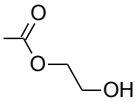
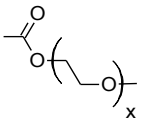
Figure 5.8. [13]C NMR of terminally olefinic cellulose acetate pent-4-enoate 3, CM product (with HEA) 3b, and hydrogenated product 3b'.

For the pent-4-enoate-based derivatives (**3a-3c**), however, heterogeneous hydrogenation (Pd/C) reduced less than 30% of the double bonds in an overnight reaction at hydrogen pressure as high as 140 psi. Given the fact that the olefins in **3a-3c** are only 3 carbons away from the cellulosic backbone, it is reasonable to attribute the failure of the heterogeneous hydrogenation to steric interference by the cellulose chain which kept the double bonds from proper interactions with the Pd/C surface.[46] To overcome this difficulty, homogeneous hydrogenation was performed on the 4-pentenoate-based derivatives using 2 wt % Crabtree's catalyst[47] or 3 mol % Wilkinson's catalyst[48] under 80 psi H_2 in THF. NMR spectra (**Figure 5.7**, **5.8** and **S10-11**) of the hydrogenated products of **3a-3c** clearly indicated successful hydrogenation in similar fashion as described for **2a-2c**. However, for **3a**, the resulting hydrogenated products could not be redissolved in THF, and were only partially soluble in DMSO. To obtain soluble hydrogenated

product **3a'**, three cycles of heterogeneous hydrogenation (Pd/C) were performed (as described in the Methods section). The product obtained in this way was readily soluble, and the double bond was fully reduced as proven by ^{1}H NMR as well as FTIR (**S10** and **S4**).

Table 5.2. Molecular weight, DS and Tg of cellulose ester CM products.



Compound (abbr. of CM partner used[a])	M_n (kDa)	DP	Đ	X	DS(X)[d]	DS(Ac)[d]	T_g (ºC)
1	38.0[b]	151	NA[b]	-	-	1.82	180
2	52.2/49.8[c, g]	150/144	1.60/1.69[c, g]	-	0.67	1.88	127
2a' (AA)	59.9	159	1.41		0.64	1.87	115
2b' (HEA)	60.4	149	1.49		0.69	1.76	102
2c' (PEGMEA)	96.7	148	1.52		NA[e]	NA[e]	NA[f]
2d' (MA)	60.6[b]	149	1.70[b]		0.68	1.69	111
3	59.9	214	1.42	-	0.56	1.80	162
3a' (AA)	60.3	198	1.60		NA[e]	1.82	154
3b' (HEA)	100.8	294	1.78		0.58	1.86	141
3c' (PEGMEA)	180.1	324	1.55		NA[e]	NA[e]	76

[a]See **Table 5.1**. [b]Data reported by supplier. [c]SEC (THF, 40 ºC) [d]Calculated by ^{1}H NMR. [e]DS cannot be calculated due to peak overlap. [f]T_g not observed by DSC or MDSC. [g]MW data for **2**

are different from corresponding sample in our previous publication (M_n~36.8 kDa, Đ~1.98)[34]; besides being prepared separately the samples were analyzed by different SEC systems.

We used SEC to characterize any change in DP resulting from the CM reaction. Considering that the CM products were prone to cross-link during storage, only hydrogenated samples were analyzed by SEC (**Table 5.2**). For 2a'-2d' and 3a', both chain scission and chain coupling (due to intermolecular self-metathesis (SM)) were negligible during CM as well as Pd/C catalyzed hydrogenation, indicated by the nearly unchanged DP and dispersity (Đ; IUPAC has discouraged use of the term *polydispersity index* (PDI), which had been used to describe polymer molecular weight distribution, and replaced it with the term *dispersity,* represented by the symbol Đ[49]). These results are consistent with our previous observations[34]. The excess of CM partner used (20 equiv) enhanced the likelihood of the cellulosic terminal double bonds meeting and reacting with a CM partner rather than with another terminal double bond, effectively suppressing SM crosslinking. The mild reaction conditions of both the CM and catalytic hydrogenation reactions, on the other hand, minimized the likelihood of chain scission, and so preserved polymer DP. However, it is noteworthy that **3b'** and **3c'**, for which we employed homogeneous hydrogenation catalysts (Wilkinson's or Crabtree's) due to incomplete hydrogenation using Pd/C, had much higher DP than their shared starting material **3**. The same phenomenon was observed upon homogeneous hydrogenation of **2b** (DP~345 compared with DP~149 for **2b'**). Combined with the fact that the homogeneous hydrogenation products of **3a** lost their solubility, it is possible that the increased DP of **3b'** and **3c'** might be due to undetermined side reactions during homogeneous hydrogenation, or physical crosslinking by the hydrogenation catalysts.

Glass transition temperature (T_g), which reflects the molecular mobility of a polymer, influences the polymer's physicochemical properties including viscoelasticity, brittleness, and physical and chemical stability. It becomes a critical parameter in applications like amorphous solid dispersion formulation[50], where polymers are used as matrices to trap drug molecules in amorphous form, thereby enabling generation of supersaturated aqueous solutions. At temperatures below the formulation T_g, the restricted molecular mobility of the polymer will prevent drug molecule migration and therefore crystallization. For this reason, polymers with T_g at least 50°C higher than ambient temperature are highly desirable to keep the formulation T_g

above ambient temperature in spite of the plasticizing effects of both drug and atmospheric moisture. We employed DSC to determine T_g values of our CM products. Elimination of the double bond by hydrogenation did not significantly affect polymer T_g (**S12**). Although all other polymers exhibited T_g values at least 50 ºC above room temperature, **2c'** did not display a detectable glass transition between -40 and 190°C either by standard or modulated DSC. This may be attributed to the relatively high DS of the PEG tail and its plasticizing effect. Polymers synthesized from **3** cellulose acetate pent-4-enoate (**3a'-3c'**) had much higher T_g values (≥ 25 ºC higher) than those of their counterparts (**2a'-2c'**) synthesized from the corresponding cellulose acetate undec-10-enoate (**2**), which is likely due to internal plasticization by the long (> 11 carbons) chains, as well as the slightly lower DS of the pentenoate substituents compared to **2a'-2c'**.

5.4 Conclusions

We provide in this study an expanded vision of a new, modular method for synthesis of cellulose derivatives that can expand the utility of conventional substitution methods like esterification and etherification. We do so by introducing the concept of attaching a handle for olefin cross metathesis by these conventional methods, followed by modular CM to introduce a plethora of new functional groups. We demonstrate that readily available acrylate esters are highly effective CM partners, providing the capability to introduce α,β-unsaturated ester moieties in controlled and selective fashion as side chains of cellulose. We show how the unsaturation can be eliminated by catalytic hydrogenation, even in a one-pot overall reaction, to remove the reactivity and instability introduced by that functional group. At the same time it is clear that the α,β-unsaturation could be used alternatively as a handle for introduction of still other functionality, e.g. by Michael addition of an amine[51]. We demonstrate also that such acrylate esters may bear terminal hydroxyl groups and still be effective CM partners (2-hydroxylethyl acrylate, poly(ethylene glycol) methyl ether acrylate). These create still more potential for functionalization by reaction with the hydroxyl group (distant from the main cellulose chain and so relatively unhindered). We predict that such functionalization could be carried out prior to the CM reaction by modifying the CM partner, or after CM and/or after the CM/hydrogenation sequence. Some limitations upon this modular CM method for functionalization of cellulose become apparent as well. The double bonds of allylic alcohols may in some cases be too self-

reactive (Type 1 by Grubbs' rules) to serve as optimal CM partners, although the 60% conversions we achieved might be perfectly useful and acceptable depending on the particular synthetic goal. In contrast, unprotected amines do not appear to be effective CM partners for these terminally unsaturated cellulose esters, most likely due to their propensity to coordinate and thus inactivate the ruthenium catalyst.

It is interesting to compare this chemistry to the definition of a polymer click chemistry reaction recently put forth in eloquent fashion by Barner-Kowollik et al[22]. Clearly the reaction occurs rapidly and under mild conditions, is chemoselective and has a single reaction trajectory, affords high yields, is modular and wide in scope, and lends itself to easy product purification, as required by the authors' definition of a polymer click reaction. It does not meet their definition in the sense that the initial products are not fully stable (though they are after the hydrogenation step), and especially in that equimolarity of reagents is not ideal for achieving high yields and selectivity for CM to the exclusion of SM. Therefore we feel that characterizing the reaction as modular and click-like is appropriate, though it does not meet all the click criteria as defined by these authors.

Overall, the mild nature of this CM chemistry, and our growing appreciation of the potential variety of CM partners that can be used, illustrate its high potential for modular modifications of terminally unsaturated cellulose derivatives. Compared with other potential "click" partners, the terminal olefins required for this "click-like" reaction are more readily accessible and can be elaborated with various functional moieties by, for example, simple esterification with acrylic acid. Moreover, the approach is very likely to be applicable to other polysaccharides as well. This example of the marriage of polysaccharide chemistry with organometallic chemistry not only illuminates multiple pathways to novel polysaccharide derivatives, but also creates a valuable platform for structure-activity relationship studies. By modular addition of a variety of CM partners (potentially containing a variety of functional group types) to a single terminally unsaturated polysaccharide derivative, a family of polysaccharide derivatives can be prepared that ***share identical Mw, DS, substitution pattern, and monosaccharide sequence, differing only in the side-chain functional group***s. This synthetic strategy will enable unambiguous investigation of structure-activity relationships with regard, for example, to different appended functional groups, thereby enriching our understanding of these attractive derivatives of natural polysaccharides.

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Chapter 6: Synthesis of amide-functionalized cellulose esters by olefin cross-metathesis

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Abstract

Cellulose esters with amide functionalities were synthesized by cross-metathesis (CM) reaction of terminally olefinic esters with different acrylamides, catalyzed by Hoveyda-Grubbs 2nd generation catalyst. Chelation by amides of the catalyst ruthenium center caused low conversions using conventional solvents. The effects of both solvent and structure of acrylamide on reaction conversion were investigated. While the inherent tendency of acrylamides to chelate Ru is governed by the acrylamide N-substituents, employing acetic acid as a solvent significantly improved the conversion of certain acrylamides, from 50% to up to 99%. Homogeneous hydrogenation using *p*-toluenesulfonyl hydrazide successfully eliminated the a, b-unsaturation of the CM products to give stable amide-functionalized cellulose esters. The amide-functionalized product showed higher T_g than its starting terminally olefinic counterpart, which may have resulted from strong hydrogen bonding interactions of the amide functional groups.

6.1 Introduction

Amide functionality on the backbone or side chain of a polymer can not only affect physical, chemical, and mechanical properties[1], but also polymer-polymer[2] or polymer-small molecule[3] interaction by secondary forces like hydrogen bonding[4-6]. Although amide-containing synthetic polymers (e.g. nylons) as well as some natural polymers such as proteins have been well studied, to date relatively few studies have focused on the synthesis and properties of amide functionalized cellulose and other polysaccharide derivatives. This is in part because of the limited number of synthetic approaches that have been described to prepare such derivatives. Amidation of carboxylic acid containing polysaccharides or their derivatives (e.g. carboxymethyl cellulose) with amines gives substituted amides; this method can be challenging since polysaccharide hydroxyl groups may also be reactive towards an activated carboxyl group[7,8]. In a

reversal of reactivity, amidation between amino-containing polysaccharides (e.g. chitosan) with carboxylic acids or acid chlorides has also been employed; the required amino substituted polysaccharide may not be readily available, and issues of N/O selectivity can also surface with this method[9].

Olefin metathesis has been proven to be a powerful tool for synthesis of a variety of both small and polymeric molecules by scission and regeneration of carbon-carbon double bonds[10]. Successful synthesis of small molecule amides through cross-metathesis (CM) has also been reported[11-14]. In 2001, the Grubbs group investigated the synthesis of a, b-unsaturated amides by cross-coupling reaction of simple terminal olefins with corresponding amides (e.g. acrylamide)[11]. Olefin CM chemistry has been adopted by the Edgar[15,16] and other groups[17,18] for polysaccharide modification towards discrete or cross-linked products. In our previous studies, cellulose esters with side-chain functionalities of carboxylic acid and a variety of carboxylate esters were synthesized by CM between ω-unsaturated cellulose esters (e.g. cellulose acetate undec-10-enoate) and CM partners including acrylic acid and the corresponding acrylates (**Figure 6.1**). This synthetic pathway has been demonstrated to be a modular and efficient route to families of novel polysaccharide derivatives, providing a valuable platform for structure-property relationship studies.

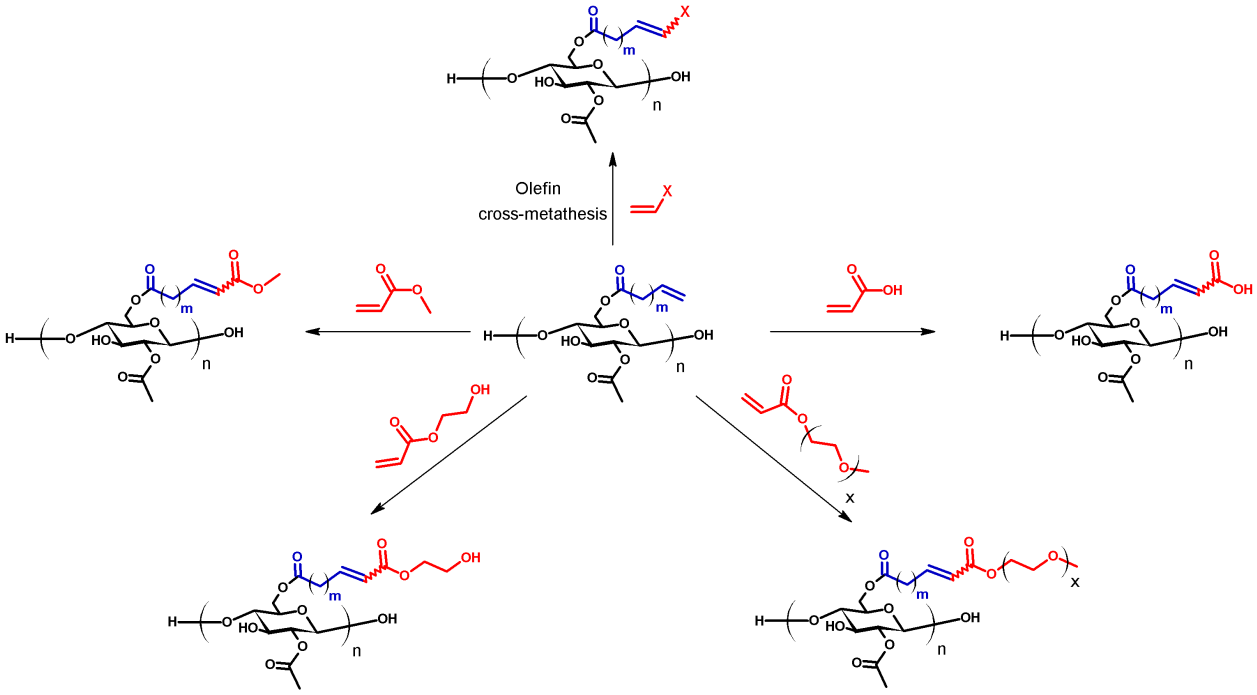


Figure 6.1. General scheme of olefin CM between terminally olefinic cellulose ester and different CM partners.

However, to the best our knowledge, there have been no reports of synthesis of α, β-unsaturated amides using olefin CM to achieve either synthetic polymer or polysaccharide modification. This may be due to the fact that amides tend to chelate with the olefin metathesis catalyst, and thus the reaction could be expected to afford even lower conversion in a polymer system than the incomplete conversions previously observed for small molecules[11]. We hypothesize that it may be possible to carry out olefin CM reactions between terminally unsaturated cellulose esters and acrylamides, so long as proper solvents or additives are used to minimize deactivation of the metathesis catalyst by the amide groups. Herein, we report our approaches to synthesis of amide-functionalized cellulose esters by cross-metathesis reaction between terminally olefinic cellulose esters and acrylamides. We also describe the impact of using organic acids (i.e. acetic acid) as solvents for CM reactions with acrylamides, and try to rationalize the observed effects via a preliminary structure-activity relationship study. Furthermore, we propose a hydrogenation protocol to eliminate instability caused by labile g-hydrogens in the initial α, β-unsaturated CM products. As mentioned earlier, intra- and intermolecular hydrogen bonding interaction via amide functionality may invest polymers with

properties of interest for applications such as amorphous solid dispersion formulation[19] and molecular recognition[3]. The current study focuses on our attempts to prove the concept of our hypothesis by developing a synthetic approach towards amide-functionalized cellulose derivatives, which may have great promise for these and other applications.

6.2 Experimental

6.2.1 Materials

Cellulose acetate (CA-320S, M_n 38.0 kDa, DS(Ac) 1.82) was from Eastman Chemical Company. The molecular weight information was reported by the supplier and the DS value was previously measured by our group[20]. Triethylamine (TEA) and 1,3-dimethyl-2-imidazolidinone (DMI) were purchased from Acros Organics. Dimethyl formamide (DMF), glacial acetic acid, ethyl acetate (EtOAc), methyl ethyl ketone (butanone, MEK) and ethyl ether were purchased from Fisher Scientific. Undec-10-enoyl chloride, Hoveyda-Grubbs 2nd generation catalyst, *p*-toluenesulfonyl hydrazide, acrylamide, *N*-phenylacrylamide, *N,N*-dimethylacrylamide, tetrahydrofuran (THF), butylated hydroxytoluene (BHT) and *N*-isopropylacrylamide were purchased from Sigma-Aldrich. DMI and DMF were dried over 4 Å molecular sieves before use. All other purchased reagents were used as received.

6.2.2 Measurements

^{1}H NMR spectra were acquired on a Bruker Avance 500 spectrometer operating at 500 MHz with 16 scans. Samples were analyzed as solutions in $CDCl_3$ or DMSO-d_6 (ca. 10 mg/mL) at 25 °C in standard 5 mm o.d. tubes. Three drops of trifluoroacetic acid were added to shift the water peak downfield from the spectral region of interest. ^{13}C NMR spectra were obtained on a Bruker Avance 500 spectrometer with a minimum of 5000 scans in DMSO-d_6 (ca. 50 mg/mL) at 25 °C. To obtain the T_g values of the cellulosic polymers, DSC was performed on a TA Instruments Q2000 apparatus using a heat/cool/heat cycle. Dry powders (ca. 5 mg) were loaded in TA hermetic aluminum pans. Each sample was equilibrated at −50 °C before heating to 160 °C at the rate of 20 °C/min, followed by quenching to −50 °C and heating to around 200 °C at a rate of 20 °C/min. FTIR spectra were obtained on a Nicolet 8700 instrument. Size exclusion chromatography (SEC), if not otherwise specified, was performed on an Agilent 1260 Infinity Multi-Detector SEC using DMAc with 0.1 M LiCl as the mobile phase (50 ºC) with 3 PLgel

10 μm mixed-B 300 × 7.5 mm columns in series. A system of multiple detectors connected in series was used for the analysis. A multi-angle laser light scattering (MALS) detector (DAWN-HELEOS II, Wyatt Technology Corporation, Goleta, CA), operating at a wavelength of 658 nm, a viscometer detector (Viscostar, Wyatt Technology Corporation, Goleta, CA), and a refractive index detector operating at a wavelength of 658 nm (Optilab T-rEX, Wyatt Technology Corporation, Goleta, CA) provided online results. Data acquisition and analysis was conducted using Astra 6 software (Wyatt Technology Corporation, Goleta, CA). Monodisperse polystyrene standard (Mw ~ 21k, Đ ~ 1.02) was run first in every sample series for the purpose of calibration and confirmation.

6.2.3 Preparation of Cellulose Acetate 10-Undecenoate (CA-Un067)

The procedure for the preparation of CA-Un067 was modified from our previously published method[16]. In detail, CA-320S (1.00 g, 4.19 mmol/AGU) was dissolved in DMI (30 mL), and the solution was heated to 90 °C with mechanical stirring under N_2. Triethylamine (1.29 mL, 9.22 mmol, 2.2 equiv.) was added; a condenser was used to avoid evaporative loss of the base catalyst. 10-Undecenoyl chloride (1.70 g, 8.36 mmol, 2.0 equiv.) was added dropwise and allowed to react at 90 °C for 20 h. The reaction mixture was then filtered, and the filtrate was precipitated in 300 mL 50:50 water/ethyl alcohol. The precipitate was redissolved in a minimal amount of CH_2Cl_2 and reprecipitated by addition to hexane. The product was washed with hexane and dried under vacuum at 40 °C. Degrees of substitution (DS) by ^{1}H NMR: DS(undec-10-enoate) (DS(Un)) 0.67, DS(acetate) (DS(Ac)) 1.88; yield: 1.27 g, 93%.

Spectral assignments using CA-Un067 as an example: ^{1}H NMR (DMSO-d_6): 1.21 (br s, $COCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2CH_2CH_2CH=CH_2$), 1.32 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH_2CH=CH_2$), 1.50 (br s, $COCH_2C\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$), 1.8-2.1(m, $COC\underline{H}_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$, and $COC\underline{H}_3$), 2.30 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}_2CH=CH_2$), 3.3-5.3 (m, cellulose backbone), 4.8-5.0 (q, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=C\underline{H}_2$), 5.7 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C\underline{H}=CH_2$). ^{13}C NMR ($CDCl_3$): 20.4 ($CO\underline{C}H_3$), 24.8 ($COCH_2\underline{C}H_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2$), 28.8 ($COCH_2CH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2CH_2CH=CH_2$), 33.6

(CO$\underline{C}$H$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2\underline{C}$H$_2$CH=CH$_2$), 114.1
(COCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH=$\underline{C}$H$_2$), 139.0
(COCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2\underline{C}$H=CH$_2$), 168.9-173.1 (C=O), 62.2 (C-6), 72.0-76.4 (C2, C3, C5), 82.3 (C-4), 100.7 (C-1).

6.2.4 Olefin cross-metathesis

Exemplary procedure for olefin cross-metathesis reaction of CA-Un067 with acrylamides in THF. CA-Un067 (100 mg, 0.188 mmol terminal olefin, 1.0 equiv.), 5 mg BHT, and 4 mL THF were charged to a 25 mL three-neck round bottom flask. The mixture was stirred and purged with N_2. After the reagents were fully dissolved, 20 equiv. of a cross-metathesis partner (e.g. acrylamide) was added into the flask, and dissolved, followed by the addition of Hoveyda-Grubbs' 2nd generation catalyst (3.6 mg, 0.03 equiv., dissolved in 2 mL of THF) which was added dropwise to the system by syringe. After stirring for 1 h under N_2 at 30 °C, the reaction was stopped by adding 3 drops of diethylene glycol monovinyl ether. The product was collected by adding the reaction mixture to 100 mL of H_2O to precipitate the product, which was collected by filtration and washed thoroughly by H_2O before being dried under vacuum at 40 °C.

Exemplary procedure for olefin cross-metathesis reaction of CA-Un067 with acrylamides in acetic acid. CA-Un067 (100 mg, 0.188 mmol terminal olefin, 1.0 equiv.), 5 mg of BHT and 4 mL of acetic acid were charged to a 25 mL three-neck round bottom flask. The mixture was stirred and purged with N_2. After the reagents were fully dissolved, a certain amount (20 equiv.) of a cross-metathesis partner (e.g. acrylamide) was added into the flask and dissolved, followed by the addition of Hoveyda-Grubbs' 2nd generation catalyst (3.6 mg, 0.03 equiv., dissolved in 2 mL of acetic acid) which was added dropwise to the system by syringe. After stirring for 1 h under N_2 at 30 °C, the reaction was stopped by adding 3 drops of diethylene glycol monovinyl ether. The product was collected by precipitating in 100 mL of H_2O (a small amount of $NaHCO_3$ was added to facilitate the precipitation process), and washed thoroughly with H_2O before being dried under vacuum at 40 °C. The product at full conversion was named as CA-Un067-Aam.

Spectral assignment using CA-Un067-Aam as an example: ^{1}H NMR (DMSO-d$_6$): 1.21 (br s, COCH$_2$CH$_2\underline{C}$H$_2\underline{C}$H$_2\underline{C}$H$_2\underline{C}$H$_2$CH$_2$CH$_2$CH=CH$_2$CONH$_2$), 1.36 (br s, COCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2\underline{C}$H$_2$CH$_2$CH=CH$_2$CONH$_2$), 1.50 (br s, COCH$_2\underline{C}$H$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH=CH$_2$CONH$_2$), 1.8-2.4(m,

$COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2CONH_2$, and $COCH_3$), 3.3-5.3 (m, cellulose backbone), 5.8 (d, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2CONH_2$, *E* configuration), 6.6 (m, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2CONH_2$, *E* configuration). ^{13}C NMR (DMSO-d_6): 20.9 ($COCH_3$), 24.8 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2CONH_2$), 28.8 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2CONH_2$), 31.6 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2CONH_2$), 125.1 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2CONH_2$), 143.3 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CH_2CONH_2$), 167.0-173.1 (C=O), 62.2 (C-6), 72.0-76.4 (C2, C3, C5), 82.3 (C-4), 100.7 (C-1).

6.2.5 Hydrogenation of the cross-metathesis product using p-toluenesulfonyl hydrazide

CA-Un067-Aam (100 mg, 0.177 mmol olefin, 1.0 equiv.), *p*-toluenesulfonyl hydrazide (164 mg, 5.0 equiv.), 10 mg of BHT, 150 μL Et_3N and 5 mL of DMF were charged to a 2 neck round bottom flask connecting with a reflux condenser. The mixture was stirred and purged with N_2 at room temperature. After the reagents were dissolved, the mixture was heated to 135 °C using an oil bath. The solution was stirred for 5 h, and then the reaction was stopped by cooling the flask in tap water. The product was collected by adding the reaction mixture slowly to 20 mL of EtOAc, and collecting the precipitate by filtration; it was then washed thoroughly using EtOAc, ethyl ether and H_2O. The product (CA-Un067-Aam-H) was then dried under vacuum at 40 °C. ^{1}H NMR (DMSO-d_6): 1.24 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CONH_2$), 1.48 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CONH_2$), 1.8-2.4 (m, $COCH_3$, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CONH_2$), 2.75-5.25 (m, cellulose backbone), 6.75 and 7.25 (br s, $COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CONH_2$). ^{13}C NMR (DMSO-$d_6$): 20.9 ($COCH_3$), 25.5 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CONH_2$), 28.9 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CONH_2$), 35.6 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CONH_2$), 174.8 ($COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CONH_2$), 169.1-173.3 (C=O), 63.0 (C-6), 72.0-76.4 (C2, C3, C5), 80.4 (C-4), 100.0 (C-1). Yield: 72 mg, 72%.

6.3 Results and discussion

Olefin cross-metathesis has been proven a useful tool for polysaccharide chemists, in which polysaccharide derivatives (e.g. cellulose derivatives) with terminally olefinic side-chain

react with different CM partners such as acrylic acid and its esters. With the help of this chemistry, we[15,16,21] and others[17] have been able to introduce a variety of functional groups onto polysaccharide backbone in a mild, modular and efficient manner. In the current study, we examined the feasibility of using acrylamides as CM partners to obtain cellulose esters bearing amide functionalities, by the reaction scheme illustrated by **Figure 6.2**. Terminally olefinic cellulose esters were used as the substrates for CM reaction, for example cellulose undec-10-enoate with DS(Un) 0.67 (CA-Un067). The alkene side chain serves as the reactive site for CM reaction. Hoveyda-Grubbs' 2nd generation catalyst (HG-2), which has been proven tolerant to many functional groups, was chosen as the catalyst.

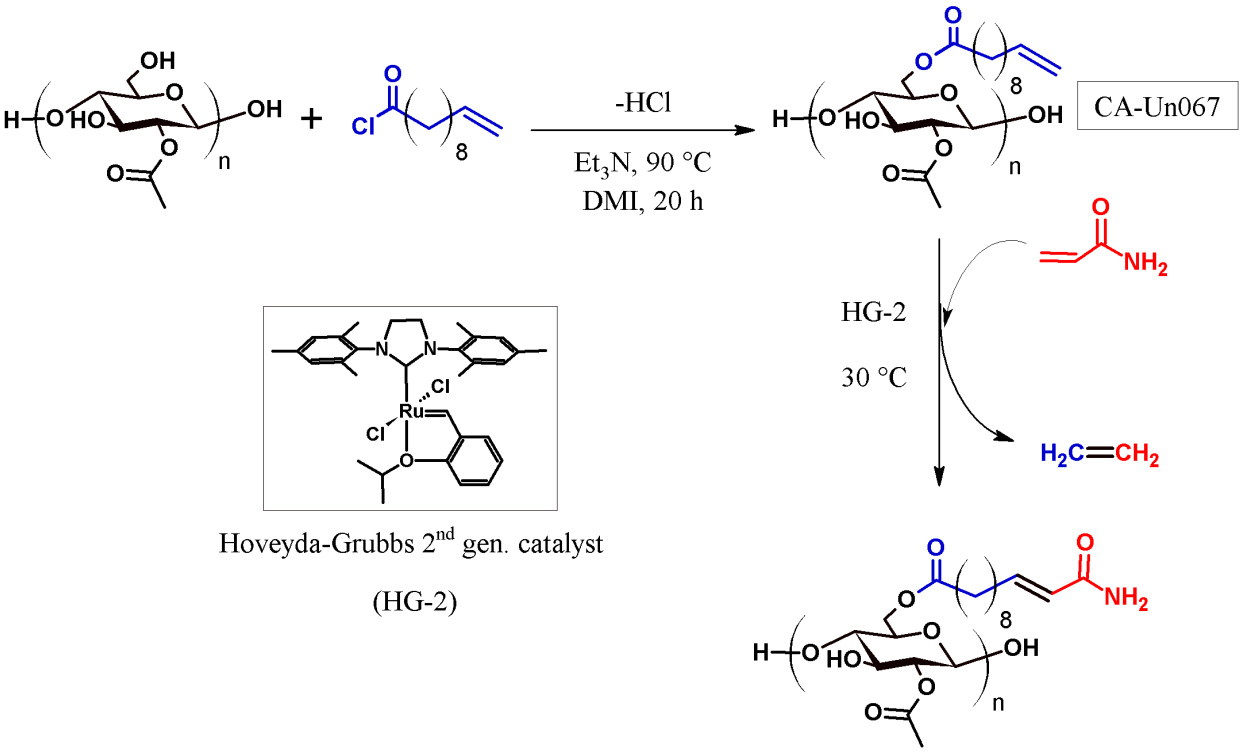


Figure 6.2. General scheme of cross-metathesis reaction of terminally olefinic cellulose ester CA-Un067 with acrylamide. Note that structures are not meant to imply regiospecificity; particular positions of substitution in all schemes are only for convenience of depiction and clarity.

We selected THF as solvent for our initial studies (**Table 6.1**), as it has been demonstrated to be useful for CM reactions of cellulose esters with a variety of partners[15,16]. Proton NMR was employed to monitor the conversion of CM reactions. As the reaction

proceeds, the terminal olefin signals of the starting polymer at around 4.90 and 5.75 ppm decrease and the signals of newly formed α,β-unsaturation at 5.84 and 6.60 ppm increase (**Figure 6.5**). In contrast to the 100% conversion achieved in CM of CA-Un067 with acrylic acid, only 15% conversion to CM product was achieved when acrylamide was employed as CM partner (**Table 6.1**, entries 1 and 2) under the same reaction conditions. Varying reaction parameters including catalyst loading, addition method, reaction time and the CM partner ratio did afford some improvement in reaction conversion, in logical fashion. For example, increasing reaction time from 1 h to 3 h improved conversion from 15% to 22%, while the conversion was 28% upon increasing catalyst loading from 3 to 6 mol%. The best conversion in THF (50%) was realized by adding a second charge of 6 mol% catalyst after 3 h (**Table 6.1**, entry 6). However, it was not possible to achieve acceptably high conversion at reasonable catalyst levels.

Table 6.1. Olefin cross-metathesis reactions of terminally olefinic cellulose ester CA-Un067 with acrylamide and acrylic acid in THF.[a]

Entry #	Cat. Loading (mol%)	CM partner	Olefin ratio[b]	Time (h)	Conversion to CM[c]
1[d]	3	acrylic acid	20	1	100%
2	3	acrylamide	20	1	15%
3	3	acrylamide	20	3	22%
4	6	acrylamide	20	1	28%
5	12	acrylamide	40	16	43%
6[e]	6+6	acrylamide	40	3+3	50%

[a]All reactions were performed at 30 ºC in THF using HG-2 catalyst. [b]Mol ratio of CM partner to olefin side chain on cellulose backbone. [c]Determined by ^{1}H NMR spectroscopy. [d]Ref. 16. [e]6

mol% of HG-2 was added into the system; after reacting for 3 h, another 6 mol% was added, then reaction was continued for an additional 3 h.

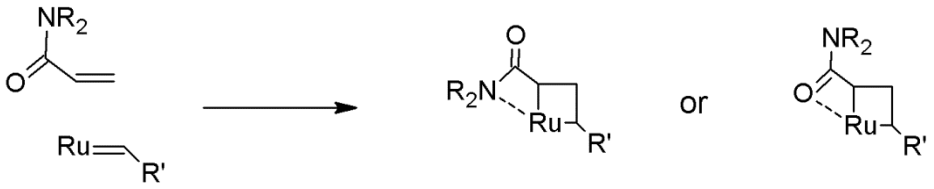


Figure 6.3. Proposed amide chelation of the catalyst in CM reactions with acrylamides.

Low conversion in CM reactions between a, b-unsaturated amides, especially electron-rich amides, and terminal olefins has been observed by several groups[11,13]. The Grubbs group suggested that for amides (especially electron-rich ones), the carbonyl group can chelate to the metal center of the catalyst and thereby decrease catalyst turnover. Such chelation effects are not unprecedented in olefin metathesis. Similar effects have also been observed during olefin metathesis of amine-containing systems[22]. Based on these studies, we believe the low observed conversion may be a result of coordination between the Ru atom of the HG-2 catalyst and the carbonyl or amine group of the amide, as shown in **Figure 6.3**. While the problematic amine group can be masked by protecting groups or by *in situ* formation of ammonium salts with the help of acid additives[13,22], this is not an option for amides, which are not sufficiently basic to readily form salts and for which protection/deprotection techniques are limited. However, based on the proposed chelation mechanism, it is reasonable to speculate that any factors that can lower the effective electron density on the amide would weaken the potential for chelation and thus increase the metathesis activity. We hypothesized that by using acetic acid as a solvent, the resulting solvation shell around the amide functional group, stabilized by H-bonding, may help minimize amide-Ru chelation and thereby enhance conversion.

Table 6.2. Olefin CM reactions of terminally olefinic cellulose ester CA-Un067 with acrylamide in acetic acid.[a]

Entry #	Cat. Loading (mol%)	CM partner	Olefin ratio[b]	Time (h)	Conversion[c]

7	6	Acrylamide	20	1	81%
8	6	Acrylamide	20	3	85%
9[d]	6+6	Acrylamide	20	1+1	94%
10[d]	6+6	Acrylamide	40	1+1	99%

[a]All reactions were performed at 30 ºC in acetic acid using HG-2 catalyst. [b]Mol ratio of acrylamide to olefin on cellulose. [c]Determined by ^{1}H NMR spectroscopy. [d]6 mol% of HG-2 was added initially; after reacting for 1 h, another 6 mol% was added and stirring continued 1 h more before the reaction was stopped.

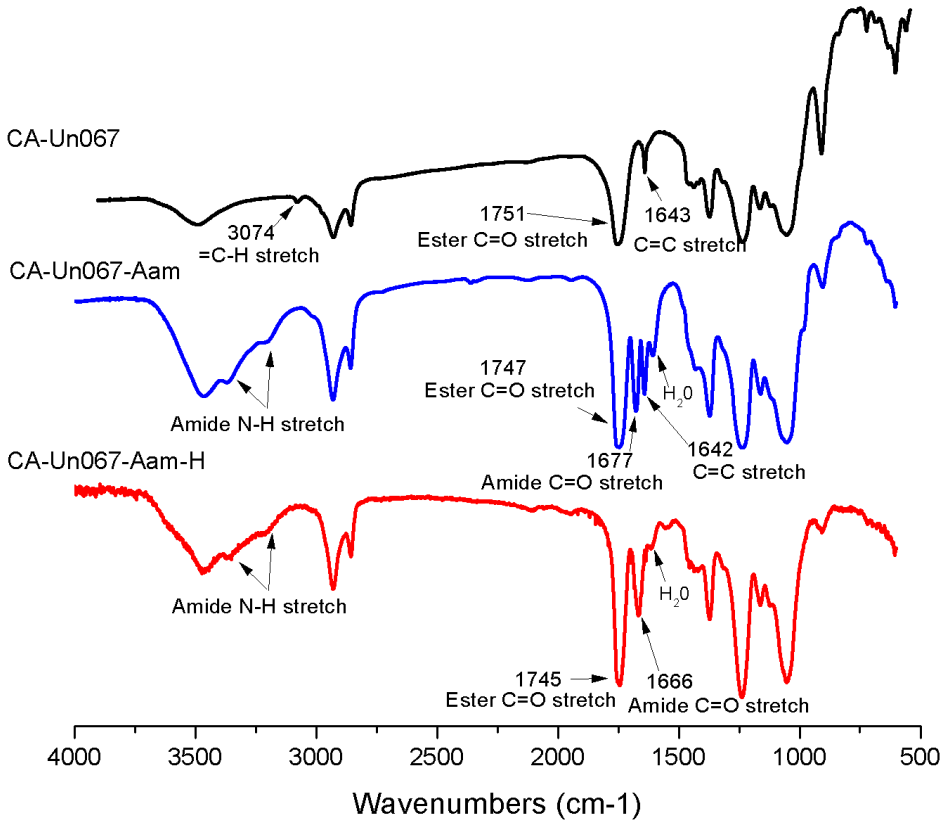


Figure 6.4. FTIR spectra of CA-Un067, CA-Un067-Aam (Entry 10, Table 2) and its hydrogenated product CA-Un067-Aam-H.

Switching from THF to acetic acid as the solvent significantly improved CM efficiency (**Table 6.2**). For example, while in THF only 28% conversion to CM was achieved for entry 4 in **Table 6.1**, 81% conversion was attained when acetic acid was used as the solvent under otherwise equivalent reaction conditions (Entry 7, **Table 6.2**; 6 mol% HG-2, 20 equiv. acrylamide, 30 ºC, 1h). Conversion of about 80% may be a perfectly acceptable result, depending on the application envisioned and on the pertinent structure-property relationship. However, for certain types of polymer post-modification, it is important to identify conditions that permit quantitative transformation; for example in some biomedical applications, where complete knowledge of the polymer structure is advantageous to obtaining regulatory approval. In pursuit of such conditions that would permit complete conversion of acrylamide, a second 6 mol% charge of catalyst was added after 60 min of reaction, and the mixture allowed to react for an additional 1 h; this methodology afforded 94% conversion. To further push the reaction towards completion, the amount of acrylamide was increased to 40 equiv., and thereby over 99% conversion was achieved. As shown in the FTIR spectra in **Figure 6.4**, the =C-H stretch peak at 3071 cm^{-1} for the terminal olefin of the start polymer (CA-Un067) completely disappeared in the spectrum of the complete conversion CM product (CA-Un067-Aam). Two shoulder peaks at 3230 and 3370 cm^{-1} were assigned to amide N-H stretch signals, while a new peak at 1677 cm^{-1}, which was assigned to amide C=O stretch, was additional evidence of successful CM reaction. More evidence was provided by proton NMR spectra. As shown in **Figure 6.5**, the terminal olefin signals at 4.90 and 5.75 ppm in the ^{1}H NMR spectrum of polymer 10 (CA-Un067-Aam) completely disappeared, while new signals corresponding to the a, b-unsaturated olefin appeared at 5.90 and 6.65 ppm (*E* configuration, no *Z* configuration signals were detected). The two broad and weak signals at 6.7 and 7.3 ppm in the spectrum of CA-Un067-Aam were assigned to amide proton resonances. The full and clean conversion achieved under these CM conditions was further proven by ^{13}C NMR spectroscopy, with the complete disappearance of terminal olefin carbon signals at 114 and 139 ppm, and the new peaks at 125 and 143 ppm corresponding to the a, b-carbons of the new olefin (**Figure 6.6**).

Cellulose esters bearing other alkanoate substituents were also tested as CM substrates. These included cellulose acetate propionate undec-10-enoate, cellulose acetate butyrate undec-10-enoate, and cellulose acetate pent-4-enoate that has a shorter olefinic side chain than its undecenoate counterparts (Supporting Information **S6.1** and **S6.2**). Gratifyingly high coupling

efficiencies (up to 95%) were also achieved with these substrates using acetic acid as solvent by procedures analogous to that for reaction of CAUn067 with acrylamide (described in the Experimental Section), showing the flexibility of this CM approach with regard to alkanoate substitution. As it has been reported that Grubbs catalysts are tolerant to many protic solvents[23,24], it would not be surprising if the approach could be extended to other polysaccharide derivatives, as long as they are soluble in solvents such as acetic acid.

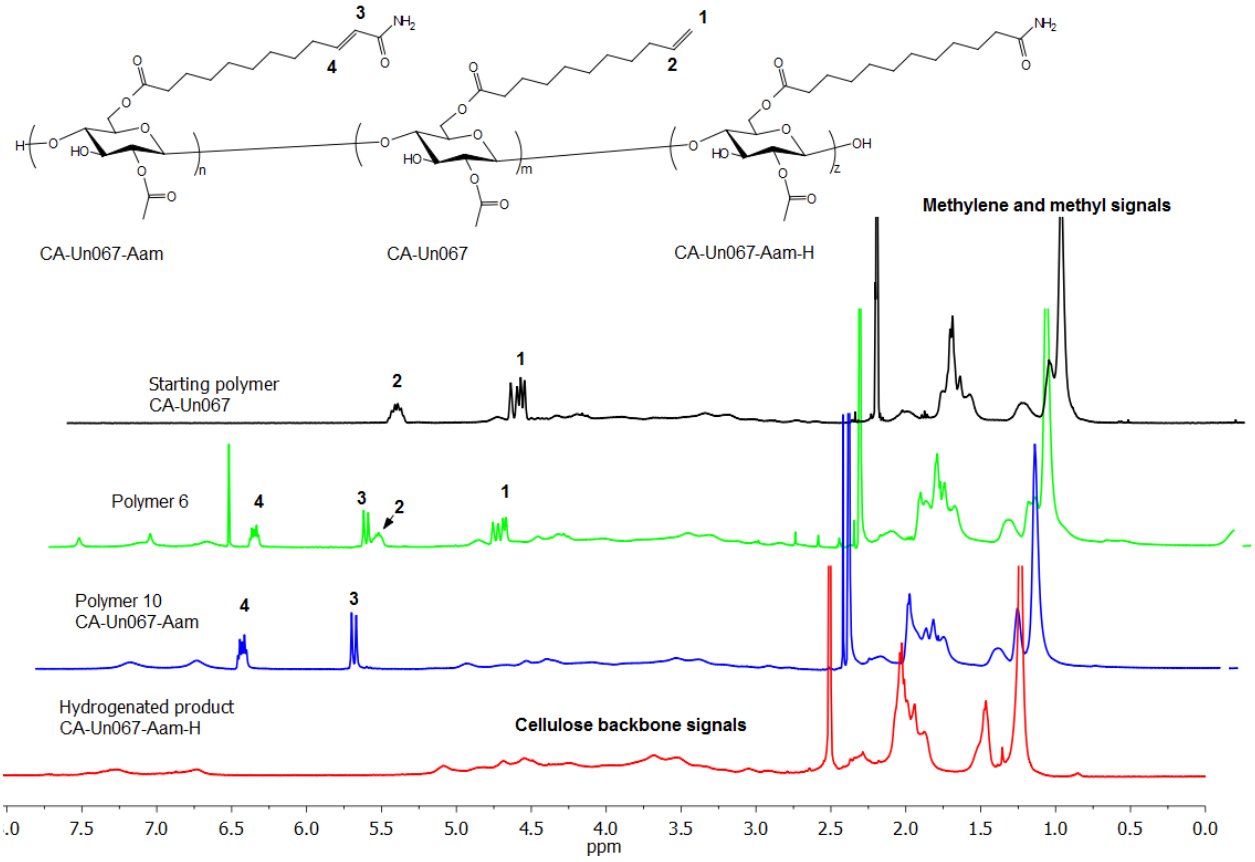


Figure 6.5. Representative ^{1}H NMR spectra of CA-Un067, incomplete CM product polymer 6, complete CM product polymer 10 (CA-Un067-Aam) and its hydrogenated product CA-Un067-Aam-H.

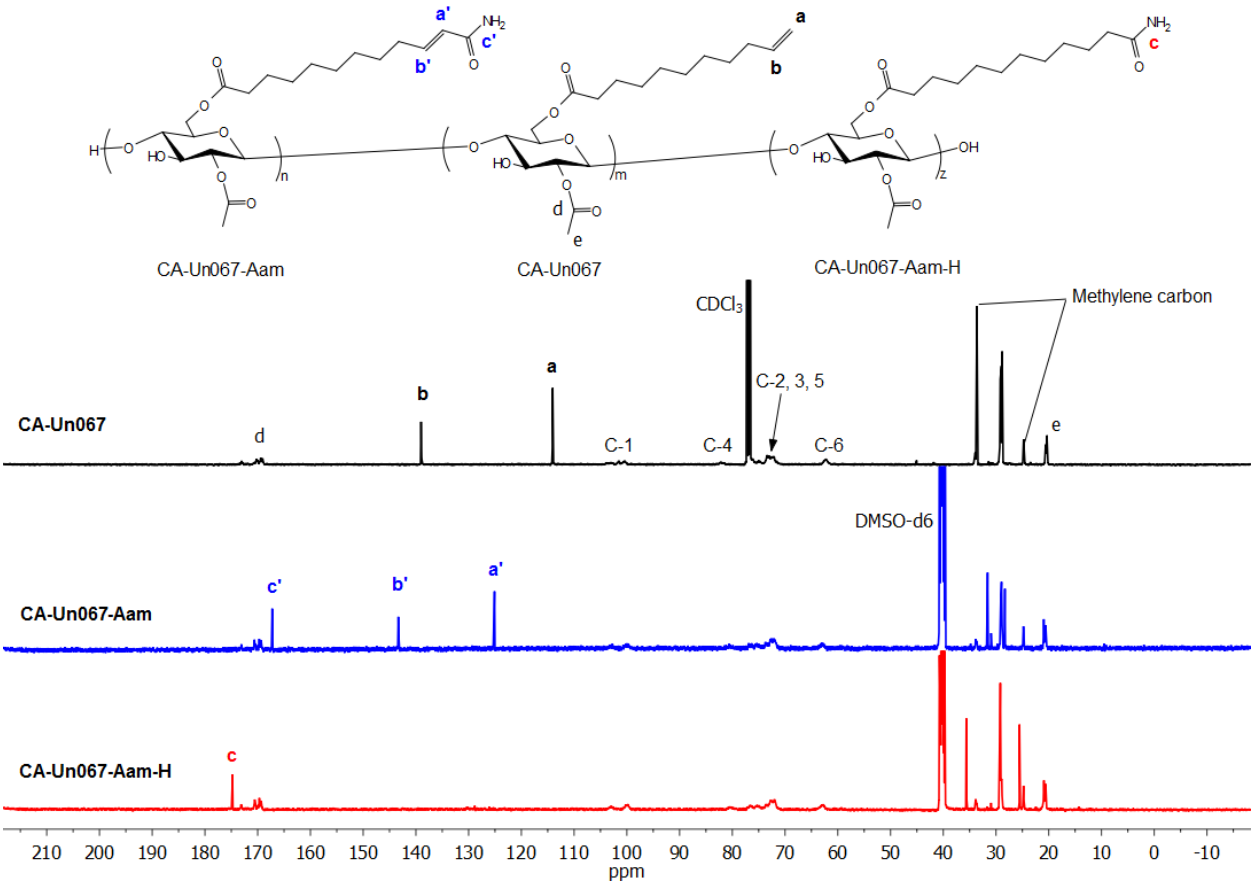


Figure 6.6. ^{13}C NMR spectra of CA-Un067, complete CM product polymer 10 (CA-Un067-Aam) and its hydrogenated product CA-Un067-Aam-H.

In our previous study[16], crosslinking was observed for CM products during storage, which we attributed to radical abstraction of g-hydrogens of the a,b-unsaturated CM product, followed by condensation reactions (e.g. radical-radical coupling, addition to double bonds) of the radicals so produced, resulting in crosslinking. The same phenomenon was also observed for the acrylamide products of this study. We reported the success of both heterogeneous and homogeneous catalytic hydrogenation methods in THF to eliminate the crosslinking potential of acrylic acid and acrylic ester CM products, by saturating the double bond[15]. Unfortunately, these methods were not useful for the amide CM polymers of this study, as they are no longer soluble in commonly used hydrogenation solvents such as THF after being isolated and dried. Hence, we sought a more universal method for hydrogenation of CM product olefins, and for that reason began to investigate diimide (generated *in situ* by thermolysis of *p*-toluenesulfonyl hydrazide) as the hydrogen source, using the more powerful solvent DMF for these amide CM products[25]. As

the CM conversions of all other polymers shown in Table 1, 2 and 3 were incomplete, only the polymer from entry 10 in **Table 6.2** (CA-Un067-Aam, conversion ~ 99%) was used for proof of concept experiments on diimide hydrogenation. As shown in **Figure 6.7**, 5 equivalents of *p*-toluenesulfonyl hydrazide in DMF completely hydrogenated CA-Un067-Aam within 5 h at 135 ºC, to give the final product (CA-Un067-Aam-H). The FTIR spectrum in **Figure 6.4** showed the disappearance of the C=C stretch, while the amide C=O stretch redshifted from 1677 to 1666 cm^{-1}, due to the elimination of the α,β-unsaturation. The proton and carbon NMR spectra in **Figure 6.5** and **Figure 6.6** showed complete disappearance of the olefin signals, confirming quantitative hydrogenation.

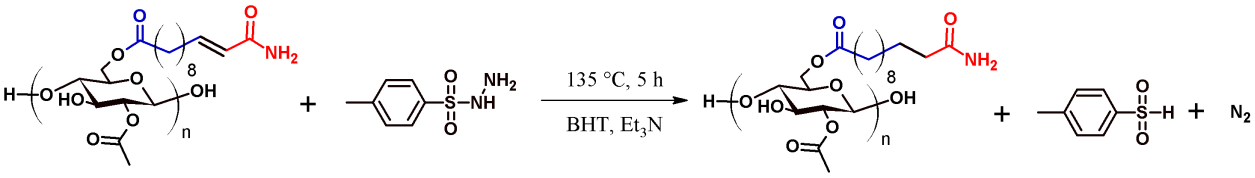


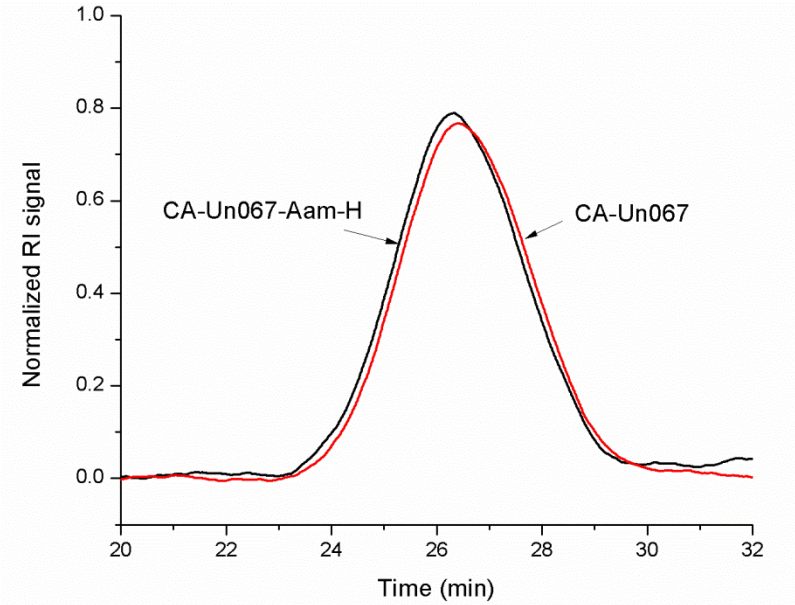
Figure 6.7. Hydrogenation of CA-Un067-Aam by *p*-toluenesulfonyl hydrazide.

As the acidic solvent (acetic acid) used in the CM reaction, and the temperature (135 ºC) in the following hydrogenation reaction are potentially detrimental to anomeric and ester linkages of the cellulose derivatives, it was necessary to examine the degree of substitution of the ester moieties and the impact on polymer DP after these reactions. From the integrals of the ^{1}H NMR spectrum of CA-Un067-Aam-H (**S3**), DS(Ac) ~ 1.90 and DS(amide) ~ 0.66. The DS(Ac) was close to its original value, indicating that the ester moieties were well preserved during CM and hydrogenation reactions.

It is quite noteworthy that after CM and hydrogenation, the molecular weight as well as PDI of the CA-Un067-Aam-H remained almost unchanged (slightly increased due to the grafting of amide functionality) compared with those of the starting cellulose ester CA-Un067, as shown in the SEC chromatograms (**Figure 6.8**). Even though the CM reaction was performed in acetic acid and the hydrogenation was performed at 135 ºC, chain scission was negligible.

It is also intriguing that in contrast with CA-Un067, which exhibits a T_g at 128 ºC, CA-Un067-Aam-H showed an endothermic DSC transition at 139 ºC, as shown in **Figure 6.9**. In our previous studies[15,16], glass transition temperatures of products after cross-metathesis reaction (with CM partners such as acrylic acid, 2-hydroxyethyl acrylate, acrylate methyl and

poly(ethylene glycol) methyl acrylate) decreased compared with T_g values of the corresponding starting terminally olefinic cellulose esters, which we described as a result of the plasticizing effect of the relatively long-chain, partly hydrophobic grafted functional group[15,26]. However, in the current study the endothermic transition we observe occurs at temperatures significantly higher than those of the previously studied cellulose alkanoate esters. As amides are known to be able to form strong intermolecular and intramolecular hydrogen bonds[6], we believe this H-bonding restricts backbone segmental motion, thus leading to higher temperature transitions.



Polymer	Mn (kDa)	Mw (kDa)	PDI	dn/dc
CA-Un067	35.4	56.5	1.59	0.0556
CA-Un067-Aam-H	38.2	63.9	1.67	0.0648

Figure 6.8. SEC traces of the starting cellulose ester (CA-Un067) and the final product after complete CM with acrylamide and hydrogenation (CA-Un067-Aam-H).

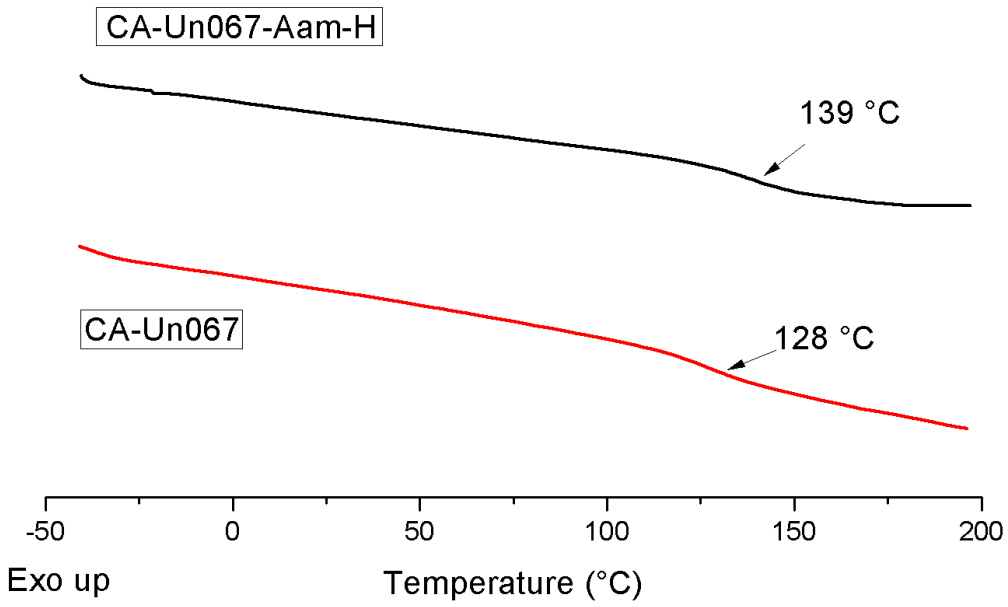


Figure 6.9. DSC analysis of the starting cellulose ester (CA-Un067) and the final product after complete CM with acrylamide followed by hydrogenation (CA-Un067-Aam-H).

With the success of CM with acrylamide in acetic acid, we wished to explore the effect of substitution at the amide nitrogen. We compared the effects of the two solvents (THF and acetic acid) on a series of acrylamide partners, including *N,N*-dimethyl acrylamide, *N*-isopropyl acrylamide and *N*-phenyl acrylamide, trying to better understand these interactions and rationalize the underlying principles. As shown in **Table 6.3**, solvents had different impacts upon CM conversion, depending on acrylamide structure. In our hypothesis, acetic acid interacts with acrylamides (likely via H-bonding) and so weakens the coordination between amide functionality and ruthenium metal center, which then contributes to increasing the conversion of the reaction. The results of CM with acrylamide and *N,N*-dimethyl acrylamide supported this hypothesis, in which using acetic acid as solvent dramatically improved CM conversion relative to reaction in THF. For CM with *N*-isopropyl acrylamide and *N*-phenyl acrylamide, acetic acid

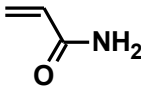
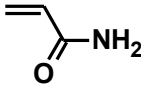
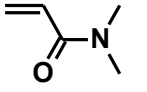
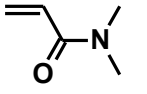
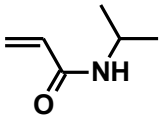
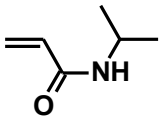
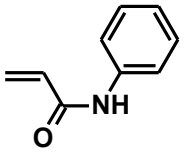
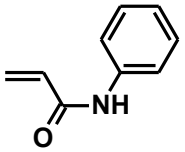
lowered the conversions, which at first seemed contradictory with regard to our previous hypothesis.

However, cross-metathesis is a complex process and the conversion to CM product is the result of multiple interactions between catalyst, solvent and CM substrates. In the case of CM with acrylamides, reaction conversion is principally influenced by two factors: the electron density of the partner (non-cellulosic) double bond, and the chelation ability of the amide. High electron density of the double bond and low chelation ability should lead to high conversion. Acrylamide *N*-substituents could affect the electron density of the double bond by electronic effects. On the other hand, they may also affect the chelation ability of the amide by changing electron density on the carboxamide oxygen and nitrogen atoms, and by steric effects (substituents with larger steric bulk may interfere with chelation with the metal center).

Taking all these factors into consideration, our results can be interpreted in a rational manner. In THF, for *N,N*-dimethyl acrylamide, the *N*-methyls are electron-donating, which increases electron density on both the nitrogen atom and the olefin. The electron density increase on nitrogen is likely more significant than that on the olefin, enhancing chelation, with the overall effect of lower conversion (39%) than that for acrylamide (50%). In the case of *N*-isopropylacrylamide, conversion as high as 82% was achieved in THF. This can be explained by the steric bulk of the isopropyl group, which weakens chelation between the amide and Ru, while the electron-donating effect is not sufficient to overcome the steric effect. For *N*-phenylacrylamide, steric hindrance as well as the electron-withdrawing influence of the phenyl substituent act in concert to minimize amide-Ru coordination, but the strong electron-withdrawing effect of the phenyl group also contributes to lowering the electron density on the olefin. These influences balance one another, and the result is 50% conversion for *N*-phenylacrylamide (entry 18), the same as achieved with acrylamide in the same solvent. When the reactions were performed in acetic acid, electron density on both olefin and nitrogen/oxygen was reduced. For acrylamide and *N*,*N*-dimethylacrylamide, the chelation effect dominates the impact upon conversion. Acetic acid solvent improved the conversion of both acrylamide and *N*,*N*-dimethyl acrylamide partners to a large degree (entries 13, 15). However, for *N*-isopropylacrylamide and *N*-phenylacrylamide, the chelation effects of which have already been minimized by the steric bulk of the *N*-substituent, running the reaction in acetic acid had its

impact mostly by reducing the olefin electron density, thereby affording lower conversion (entries 17, 19).

Table 6.3. Olefin cross-metathesis reactions of terminally olefinic cellulose ester CA-Un067 with different acrylamides in acetic acid and THF.[a]

Entry #	CM partner	Solvent	Conversion[b]
12		THF	50%
13		HOAc	95%
14		THF	39%
15		HOAc	68%
16		THF	82%
17		HOAc	73%
18		THF	50%
19		HOAc	37%

[a]All reactions were conducted under same conditions: 50 mg CA-Un067 (0.094 mmol olefin, 1 equiv.), 3 mg BHT; CM partner: 40 equiv.; catalyst: 6 mol% + 6 mol% HG2; reaction time: 1 h

+ 1 h; temperature: 30 ºC. [b]Conversion determined by proton NMR (Supporting Information S4, S5 and S6).

6.4 Conclusions

Acrylamides as cross-metathesis partners generally afforded low conversion to CM products in neutral solvents such as THF, due to chelation between the amide functional group and the ruthenium metal center. Employing acetic acid as solvent for CM reactions of terminally olefinic cellulose esters had rationally explicable effects on improving CM conversion of acrylamides. For acrylamides with small substituents such as acrylamide and *N*,*N*-dimethylacrylamide, acetic acid significantly improved the CM conversion of these partners. We rationalize this effect as resulting from the fact that acetic acid can interact with the amide through H-bonding, thereby weakening chelation of Ru by the amide. For acrylamides with large and/or electron-withdrawing substituents such as *N*-isopropylacrylamide and *N*-phenylacrylamide, low electron density on oxygen and nitrogen atoms of the amide and large steric bulk of the substituent(s) reduce amide chelation of Ru in either solvent. In these cases, using acetic acid as a solvent could not further improve the conversion, but might rather decrease the CM reactivity of the corresponding acrylamide by reducing olefin electron density. We believe that this methodology may prove generally useful for enhancing conversion of CM reactions of properly chosen acrylamides with other polysaccharides or other polymers containing appropriately designed olefinic substituents.

We also found an effective method for eliminating the unsaturation of the amide CM products of cellulose esters, by hydrogenation with diimide generated *in situ* by thermolysis of *p*-toluenesulfonyl hydrazide. This method is of particular importance for polymers that are not compatible with other catalytic hydrogenation methods, either due to solubility or reactivity (in some cases it can be difficult for rigid polysaccharide derivatives, especially those with bulky substituents, to interact effectively with the catalyst surface of a heterogeneous catalyst). We believe that this diimide hydrogenation method may prove to be of general utility for reducing the unsaturation of polysaccharide CM products. The conditions of the CM reaction in acetic acid as well as those of the hydrogenation were so mild that no significant molecular weight change or alkanoate hydrolysis was observed. The amide-functionalized cellulose esters showed thermal transitions that occurred at higher temperatures than those observed for cellulose long-

chain alkanoate derivatives. We postulate that H-bonding between amide substituents likely restricted segmental motion of the cellulose main chain and resulted to higher glass transition temperature.

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Chapter 7: Multifunctional cellulose esters by olefin cross-metathesis and thiol-Michael addition

(Adapted with permission from the Royal Society of Chemistry from Meng X., Roy Choudhury S. and Edgar, K. J. Multifunctional cellulose esters by olefin cross-metathesis and thiol-Michael addition. Polym. Chem. 2016, 7, 3748-3856)

Abstract

Olefin cross-metathesis (CM) has been shown to be a versatile, mild, modular and efficient approach to polysaccharide modification. One issue with regard to this approach is the susceptibility of the initial α,β-unsaturated CM derivatives to H-atom abstraction in the γ-position, followed by radical recombination that leads to insoluble, crosslinked products. In our original approach, we resolved this problem by removing the offending unsaturation via hydrogenation. In the current study, we describe a method to exploit these reactive conjugated olefins, by post-CM thiol-Michael addition, thereby appending additional functionality. CM substrates and thiols bearing various functional groups were combined and reacted, employing amine catalysis. Up to 100% conversion was achieved under optimal conditions (e.g. catalyst and reaction time), with minimal side reactions observed. The combination of the two modular reactions creates versatile access to cellulose derivatives equipped with a wide diversity of functional groups.

7.1 Introduction

Thiol-Michael addition is the 1,4-nucleophilic conjugate addition of a thiol anion to an α,β-unsaturated carbonyl (hydrothiolation) usually in the presence of a base or nucleophile catalyst. In thiol-Michael reactions, the addition of thiols to electron-deficient carbon-carbon double bonds can be achieved rapidly, quantitatively and orthogonally under mild conditions. Furthermore, unlike thiol-ene reactions in which radical-radical termination occurs from time to time due to the free radical nature of the chemistry, thiol-Michael additions typically are not accompanied by significant side reactions[1]. Due to these merits, the thiol-Michael addition is now thought of as a "click" reaction, and has been broadly implemented in polymer and polysaccharide synthesis and modification[2-5].

Typically, in order to employ thiol-Michael reactions in polysaccharide modification, two major strategies have been used; a) thiolation of the polysaccharide backbone to afford a polymeric thiol reagent, or b) introduction of electron-deficient acceptor olefins to polysaccharides.[6] Although both of the approaches have been extensively explored, polythiols are capable of forming intra- and intermolecular crosslinks via disulfide bond formation[7,8]. Unless the networks are the desired products, this may become a problem in polymer preparation and storage. Attachment of an acceptor activated olefin to the polysaccharide, on the other hand, may avoid such problems, and such olefin functionality can be a handle either for crosslinking or "clicking" small molecules by thiol-ene/thiol-Michael reactions[9].

Direct modification of cellulose derivatives bearing pendent alkenes has been reported by several groups. Schumann et al.[10] and Meng et al.[11] employed hydroboration-oxidation sequences to convert terminal olefins on cellulose ethers and esters, respectively, to w-hydroxyl groups. Thiol-ene reactions have also received tremendous interest in cellulose functionalization.[6] Researchers have taken advantage of its facility and simplicity to apply thiol-ene reactions to cellulose surface modification[12,13], homogeneous functionalization[14], and synthesis of crosslinked composites[15]. Recently, the Edgar group successfully applied olefin CM to polysaccharide modification, in which cellulose esters and ethers with w-unsaturated side chains were reacted with different CM partners such as acrylic acid[16], acrylates[17,18] and acrylamides[19] employing the Hoveyda-Grubbs 2nd generation Ru catalyst to afford a variety of side-chain functionalized cellulose derivatives under mild conditions. This is a mild, modular, versatile, and rapid method for polysaccharide post-modification. Several recent reviews[6,20,21] on olefin metathesis and olefin cross-metathesis have described the utility of these methods for polymerization and other synthetic applications.

CM between ω-unsaturated polysaccharide side-chains and different CM partners affords a, b-unsaturated carboxylic acids, esters and amides. Given that a, b-unsaturated carbonyls are potentially good substrates for thiol-Michael addition, and since it is essential in any case to eliminate the a, b-unsaturation in order to stabilize the derivatives against radical-initiated dimerization, it is tempting to combine CM with the thiol-Michael addition. We hypothesize that, under mild conditions, a variety of activated olefin derivatives that result from modular CM reaction can react with a series of thiols via the thiol-Michael click reaction to provide polysaccharides bearing functional side-chains that may possess even greater diversity. Recently,

Winkler and Meier[22] reported the reaction of an α,β-unsaturated ester (from CM) with 2-mercaptoethanol or methyl thioglycolate via thiol-Michael addition to obtain AB- or AA-type of monomers for polyesterification. However, we are unaware of any such strategy being reported for either polymer post-modification or polysaccharide modification. We describe herein, our efforts to demonstrate proof of concept for such post-modification of CM products from cellulose esters.

7.2 Experimental

7.2.1 Materials

Cellulose acetate (CA-320S, M_n ~ 38 kDa, DP ~ 151 (data reported by supplier), DS_{Ac} = 1.82 (measured by ^{1}H-NMR spectroscopy)) was from Eastman Chemical Company. Triethylamine (TEA), 1,3-dimethyl-2-imidazolidinone (DMI), and dimethylsulfoxide (DMSO) were purchased from Acros Organics. Anhydrous tetrahydrofuran, acrylic acid, [2-(acryloyloxy)ethyl]trimethylammonium chloride (80 wt% in H_2O), benzyl acrylate, 2,6-di-*tert*-butyl-4-methylphenol (butylated hydroxytoluene, or BHT), 2-hydroxyethyl acrylate, Hoveyda-Grubbs II generation catalyst, n-hexylamine (HA), 2-mercaptoethanol (2-ME), 3-mercaptopropionic acid (3-MPA), cysteamine (Cys), pent-4-enoyl chloride, and undec-10-enoyl chloride were purchased from Sigma Aldrich. Diethylene glycol monovinyl ether was purchased from TCI. DMI was dried over 4 Å molecular sieves. All other purchased reagents were used as received.

7.2.2 Measurements

^{1}H NMR spectra were acquired on Bruker Avance 500 spectrometers operating at 500 MHz. Samples were analyzed as solutions in DMSO-d6 (ca. 10 mg/mL) at 25 ℃ in standard 5 mm o.d. tubes. Three drops of trifluoroacetic acid were added to shift the water peak in DMSO-d6 downfield from the spectral region of interest. ^{13}C NMR and ^{1}H-^{13}C HSQC spectra were obtained on a Bruker Avance 500 MHz spectrometer with a minimum of 5000 scans in DMSO-d6 (ca. 50 mg/mL) at 80 ℃. To obtain the T_g values of the cellulosic polymers, DSC was performed on a TA Instruments Q100 apparatus and TA Discovery DSC using heat/cool/heat mode. Dry powders (ca. 5 mg) were loaded in Tzero^{TM} aluminum pans. The scanning conditions were set as follows: each sample was equilibrated at 35 °C, and then heated to 150° at 20°C/min.

The sample was then cooled at 100 °C/min to −50°C. During the second heating cycle the sample was heated to 200°C at 20°C/min. If the heat/cool/heat mode failed to give a clear transition, modulated DSC was performed as follows: each sample was equilibrated at -50°C, the underlying ramp heating rate was 7°C, the oscillation amplitude was ± 1°C, and oscillation period was 40 s. FTIR spectra were obtained on a Nicolet 8700 instrument. Size exclusion chromatography (SEC), if not otherwise specified, was performed on Agilent 1260 Infinity Multi-Detector SEC using DMAc with 0.05 M LiCl as the mobile phase (50 ºC) with 3 PLgel 10 μm mixed-B 300 × 7.5 mm columns in series. A system of multiple detectors connected in series was used for the analysis. A multi-angle laser light scattering (MALS) detector (DAWN-HELEOS II, Wyatt Technology Corporation, Goleta, CA), operating at a wavelength of 658 nm and a refractive index detector operating at a wavelength of 658 nm (Optilab T-rEX, Wyatt Technology Corporation, Goleta, CA) provided online results. Data acquisition and analysis was conducted using Astra 6 software (Wyatt Technology Corporation, Goleta, CA). Monodisperse polystyrene standard (Mw~21k, Đ~1.02) was run first in every sample series for the purpose of calibration and confirmation.

7.2.3 Preparation of cellulose acetate undec-10-enoate (CA-Un067).

The synthesis of CA-Un067 was performed following a procedure[16,17] previously reported by our group. In detail, CA-320S (1.00 g, 4.19 mmol/AGU) was dissolved in DMI (30 mL), and the solution was heated to 90 °C in a three-neck round bottom flask equipped with condenser, with mechanical stirring under N_2. Triethylamine (1.29 mL, 9.22 mmol, 2.2 equiv.) was added, and then undec-10-enoyl chloride (1.70 g, 8.36 mmol, 2.0 equiv.) was added dropwise. The resulting solution was stirred at 90°C for 20 h. The reaction mixture was then filtered, and the filtrate was precipitated in 300 mL 50:50 water/ethyl alcohol. The precipitate was recovered by filtration, then redissolved in a minimal amount of CH_2Cl_2 and reprecipitated in hexane. The product was washed with hexane and dried under vacuum at 40°C.

A similar procedure was employed in the synthesis of cellulose acetate pent-4-enoate (CA-Pen079).

7.2.4 General procedure for olefin cross-metathesis reactions

Cross-metathesis reactions were performed following a procedure[16,17] previously reported by our group. In detail, to a flask charged with cellulose derivative CA-Un067 or CA-Pen079 (100 mg, 1.0 equiv. olefin), 5 mg BHT and 5 mL anhydrous THF were added. After the reagents were completely dissolved, cross-metathesis partner (acrylic acid, benzyl acrylate, 2-hydroxylethyl acrylate; 20 equiv.) was added followed by the addition of Hoveyda-Grubbs Catalyst 2nd Generation (0.05 equiv. in 2 mL THF) via syringe. After stirring for 1h under N_2 at 40°C, the reaction was stopped by adding 1-2 drops of diethylene glycol monovinyl ether or ethyl vinyl ether. The product was collected either by dialysis and freeze-drying, or by precipitating in H_2O/ethanol followed by sufficient washing by H_2O before being dried under vacuum at 40°C.

For the CM reaction with [2-(acryloyloxy)ethyl]trimethylammonium chloride), acetic acid was used as a solvent but otherwise the general procedure was used.

7.2.5 General procedure for thiol-Michael addition

CM product (50 mg) was dissolved in 2 mL of DMSO in a 10 mL three-neck flask. After stirring under N_2 for 30 min, the designated amount of a thiol species and a catalyst (either triethylamine or HA) was added and the solution was stirred for 5 h at room temperature. The mixture was then dialyzed against H_2O for 3 d, and then the solid product was isolated by freeze-drying.

7.3 Results and discussion

CM between an isolated, terminal olefin (Type 1 by the Grubbs classification[23]), and an electron deficient olefin (e.g. an acrylate, Type 2 by the Grubbs classification) leads to an α,β-unsaturated carboxylic acid derivative, which may serve as a substrate for thiol-Michael addition reaction. The polymer, already functionalized via CM, can thus be further modified with a secondary functional group, as shown in **Figure 7.1**.

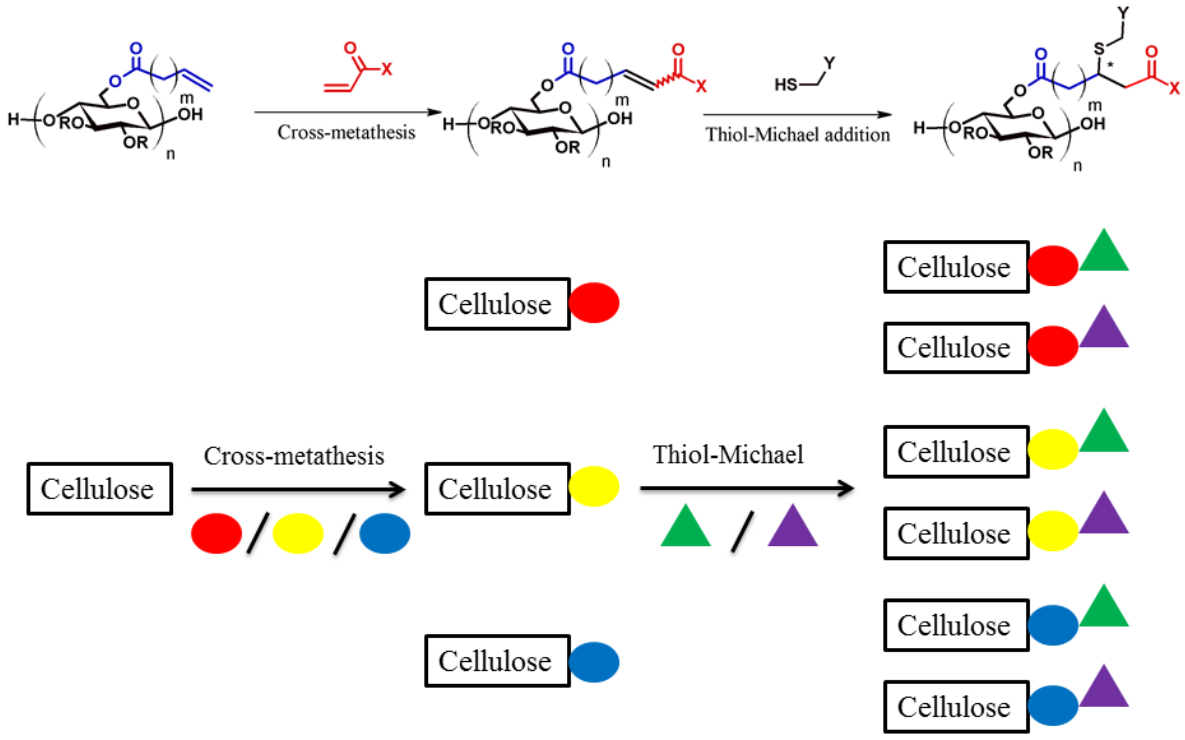


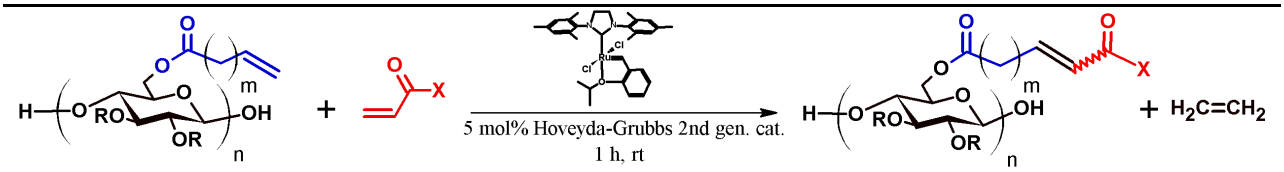
Figure 7.1. Schematic illustration of olefin cross-metathesis and thiol-Michael addition towards functionalized cellulose derivatives. *X* and *Y* in the reaction scheme represent different functional groups. The circles of different colors represent different functionalities introduced by CM, and the triangles of different colors represent different functionalities introduced by thiol-Michael addition.

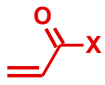
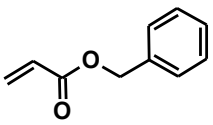
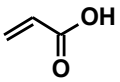
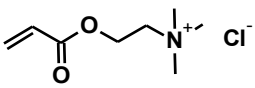
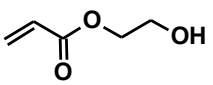
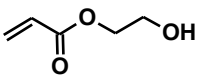
As revealed in our previous publications[16-19], CM in polysaccharide modification has a "click-like" profile and gives modular products. Using the procedure reported by Meng et al.[17], cellulose esters with terminally olefinic side-chains of different chain lengths, i.e. cellulose acetate undec-10-enoate with DS(undecenoate) 0.67 (CA-Un067) and cellulose acetate pent-4-enoate with DS(pentenoate) 0.79 (CA-Pen079), were prepared and used as the metathesis substrates. CM reaction of the starting cellulose esters with partners including acrylic acid (AA), benzyl acrylate (BA) and 2-hydroxyethyl acrylate (HEA) were performed to give full conversion

to CM products bearing a variety of functional groups, as substrates for subsequent thiol-Michael addition reaction (**Table 7.1**, polymer **1**, **2**, **4**, and **5**). In order to ensure complete conversion of reactive Type I terminal olefins to CM products without competing self-metathesis, we used 20 eq. of small molecule acrylate per eq. terminal olefin (note however that the excess acrylate is unaltered by the CM conditions and could in principle be recycled).

Positively charged cellulose ester (**3**) was also synthesized by CM reaction between CA-Un067 and CM partner [2-(acryloyloxy)ethyl]trimethylammonium chloride (TMACl, 80 wt% in H_2O). As TMACl is not miscible with typical olefin metathesis solvents such as THF and dichloromethane, we employed acetic acid as the solvent in this reaction. Acetic acid was shown to be an effective cross-metathesis solvent in our previous study of CM reactions with acrylamides[19]. As in the reactions with other CM partners, full conversion with TMACl was achieved after 1 h under the mild standard reaction conditions.

Table 7.1. Olefin cross-metathesis of ω-unsaturated cellulose esters with different CM partners.



Polymer #	Starting cellulose ester	 (20 eq.)[a]	Product abbr.[b]	Conversion[c]
1	CA-Un067 (m = 8)		CA-Un067-BA	100 %
2	CA-Un067		CA-Un067-AA	100 %[d]
3	CA-Un067		CA-Un067-TMA	100 %
4	CA-Un067		CA-Un067-HEA	100 %[b]
5	CA-Pen079 (m = 2)		CA-Pen079-HEA	100 %[b]

[a]20 mole of acrylic compound per 1 mole of double bond. [b]The rules for polymer abbreviation are explain in reference 24[24]. [c]Conversions were calculated by ^{1}H NMR. [d]As previously measured[17].

The variety of CM products synthesized was designed to explore the influences of functional group type and side-chain length upon the thiol-Michael addition. Functional groups like hydroxyl, carboxylic acid and trimethylammonium are of great interest especially in biomedical applications such as drug delivery[25], antimicrobial materials[26], and biomedical engineering[27], as they may enhance bioactivity and other performance attributes of polysaccharides. The benzyl group, on the other hand, is included since it may serve as a protecting group that enables introduction of additional functionality, so was grafted onto cellulose (CA-Un067-BA, **1**) via CM in this study. Another impetus for including a benzyl acrylate substrate is that, unlike the ^{1}H-NMR spectra of other CM adducts, the benzyl ring peaks in the spectra of CA-Un067-BA and its Michael adducts are well separated from the cellulose backbone signals (e.g. see **Figure 7.3**). As a result, the progress and conversion of the reactions can be easily monitored by integrating this peak with other corresponding peaks. For this reason, reactions with CA-Un067-BA were studied first.

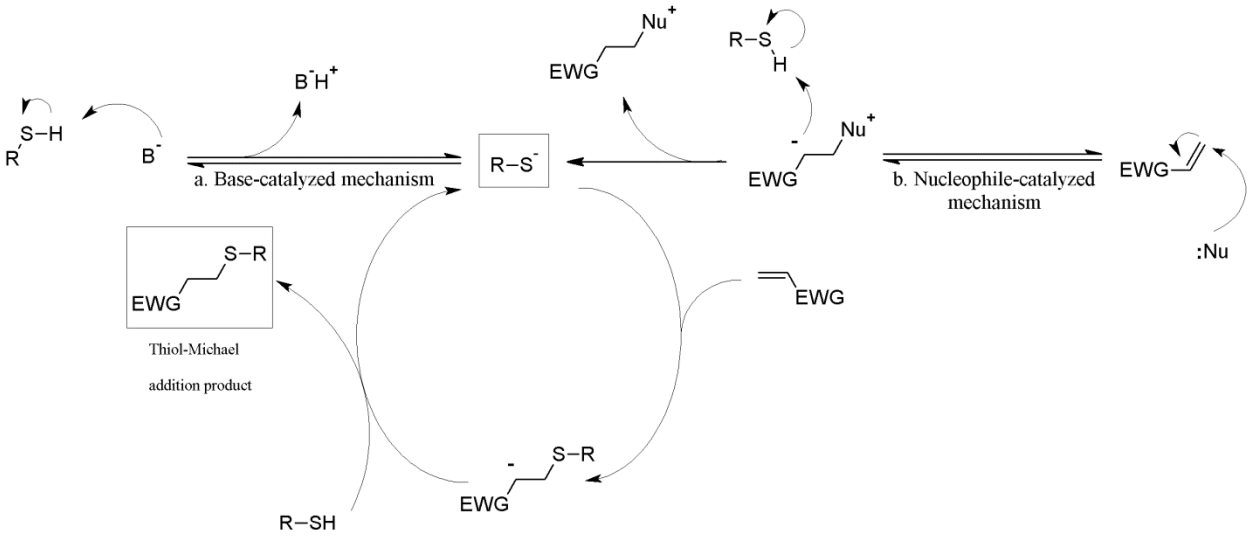
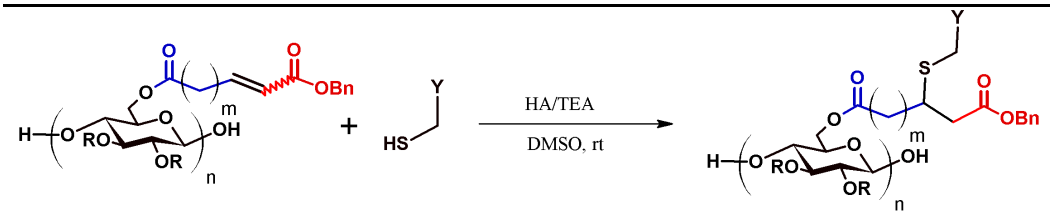


Figure 7.2. Base- and nucleophile-catalyzed mechanisms of the thiol-Michael addition reaction.

Three thiol species: 2-mercaptoethanol (2-ME), 3-mercaptopropionic acid (3-MPA) and cysteamine (CA), were selected as representative thiol-Michael partners, because they are uncharged, negatively charged, and positively charged at near-physiological pH values,

respectively. Although a variety of types of thiol-Michael catalysts, and catalytic mechanisms including base- and nucleophile-catalyzed pathways have been proposed[1], in the current study we chose to focus only on TEA and HA, arguably representing primary catalysis by base and nucleophile, respectively (**Figure 7.2**).

Table 7.2. Thiol-Michael addition of different thiols with CM product CA-Un067-BA(**1**).



Polymer #	**Thiol**	**Catalyst**	**Time (h)**	**Conversion[a] (%)**	**Side reaction**
6	2-ME (3 eq.)	TEA (6 eq.)	12	35	No
7	2-ME (2 eq.)	HA (0.3 eq.)	5	100	Minimal
8	3-MPA (9 eq.)	TEA (18 eq.)	12	50	No
9	3-MPA (2 eq.)	HA (3 eq.)	5	87	Yes
10	Cys (6 eq.)	TEA (3 eq.)	12	NA[b]	Cross-linked
11	Cys (2 eq.)	HA (0.3 eq.)	5	NA[b]	Cross-linked

[a]Conversions were calculated by ^{1}H NMR. [b]Conversion not available due to crosslinking.

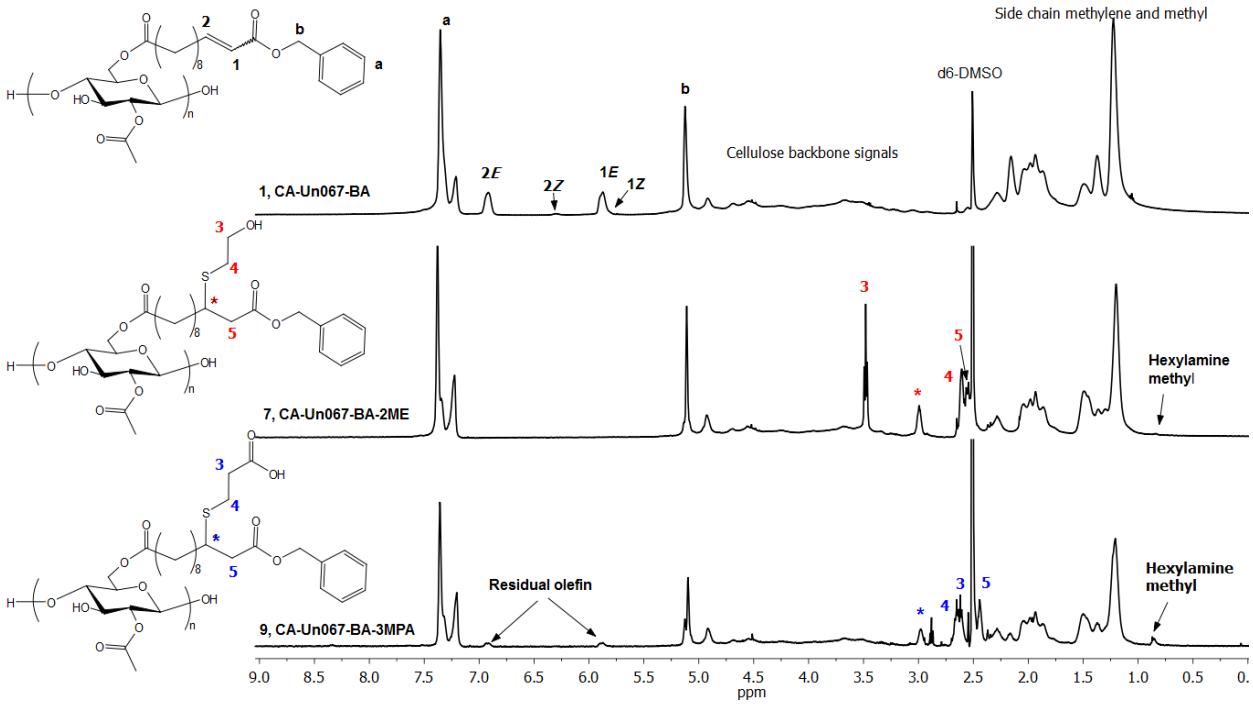


Figure 7.3. ^{1}H NMR spectra of CA-Un067-BA and its thiol-Michael products with 2-ME (CA-Un067-BA-2ME) and 3-MPA (CA-Un067-BA-3MPA).

HA proved to be more effective in catalyzing the thiol-Michael reaction of CA-Un067-BA with both 2-ME and 3-MPA (**Table 7.2**). With 2 eq. of 2-ME and 0.3 eq. of HA, complete conversion of olefin to thiol-Michael adduct (**Table 7.2**, polymer **7**, CA-Un067-BA-2ME) was achieved, as indicated by the complete disappearance of olefin proton signals at 5.87 and 6.92 ppm in the ^{1}H NMR spectrum in Figure 3, while the other related proton signals designated H3, H4, H5 and H* appeared at 3.47, 2.61, 2.57 and 2.99 ppm. Carbon NMR spectra also showed complete conversion (Supporting Information **Fig. S7.1** and **S7.2**). Reaction completion was also confirmed by FTIR spectroscopy, by disappearance of the alkene C=C stretch at 1653 cm^{-1}. It should be noted that the 1,4-conjugate addition is not expected to be chirally selective under the conditions described here, and thus in the afforded Michael adduct CA-Un067-BA-2ME, carbon * is expected to be racemic, assuming no net influence from the distant chiral centers of the cellulose backbone[28].

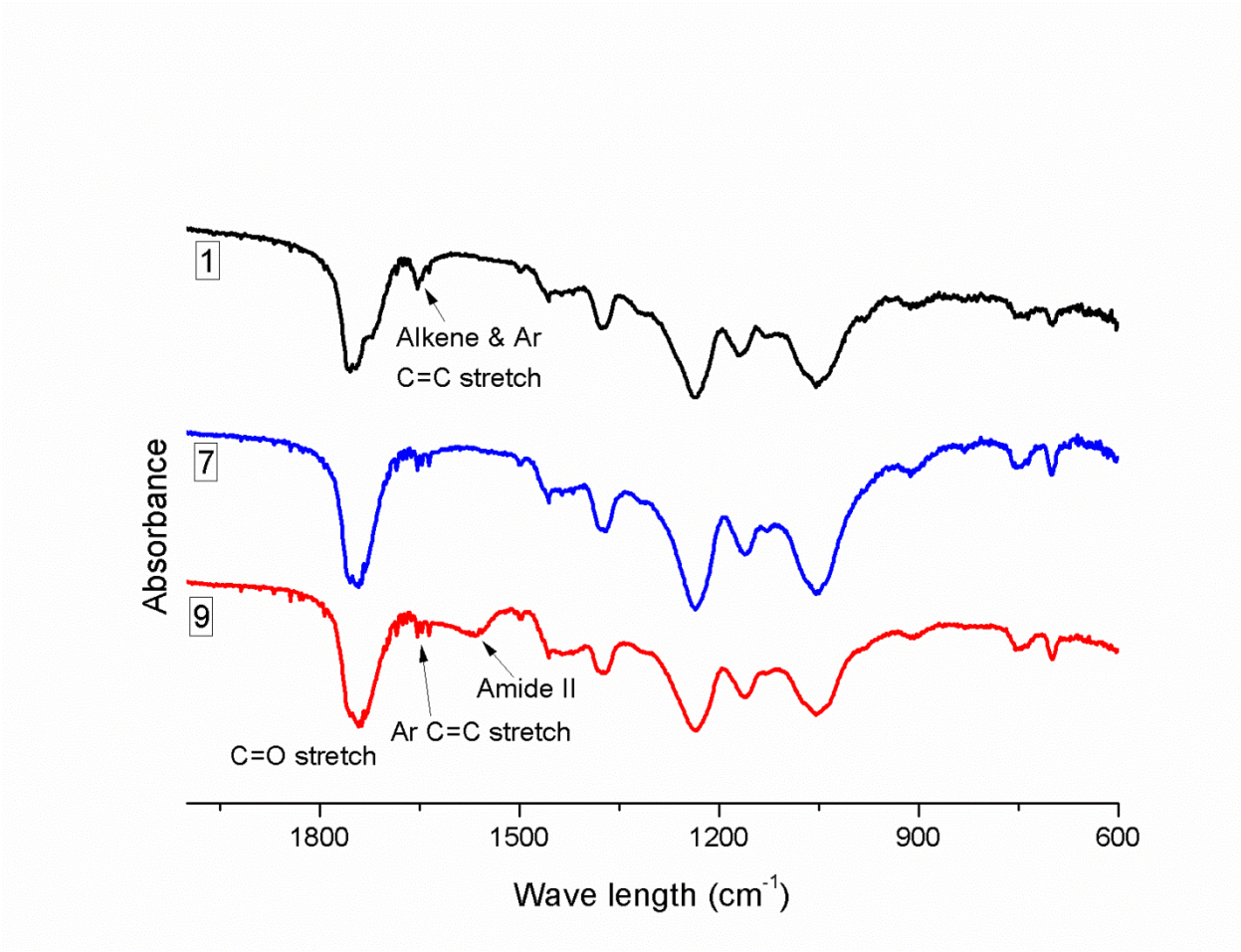


Figure 7.4. FTIR spectra of CA-Un067-BA (**1**) and its thiol-Michael products with 2-ME (CA-Un067-BA-2ME, **7**) and with 3-MPA (CA-Un067-BA-3MPA, **9**).

We noticed that the proton NMR spectrum of CA-Un067-BA-2ME contained a small peak at 0.86 ppm, in the area where we would expect the methyl group of HA to resonate. This was an indication of possible side reactions with HA. Li et al.[29] have reported that primary amines may compete with thiols in the Michael addition reaction, leading to amine adducts, especially when the amine is in large excess. We conducted reactions with higher ratios of HA to activated olefin (olefin/2-ME/HA ratio equals to 1/6/6, **Fig. S7.4**), and indeed observed a larger peak at 0.86 ppm. A primary amine acts as both base and nucleophile, and thus can catalyze both base and nucleophile hydrothiolation pathways. From this point of view, it is not unexpected to observe the 1,4-conjugate addition of HA to α,β-unsaturated carbonyls, both as a means to initiate thiol-Michael addition, and also as a side reaction in which amine competes with thiol. A

preliminary kinetic study (**Figure 7.5**) employing the same conditions as in polymer **7** (olefin/2-ME/HA ratio equals to 1/2/0.3) showed that the double bonds were consumed within 5 h, while a small peak at 0.86 ppm appeared in each spectrum from the 45 min sample to the 300 min sample. However, it is interesting that after all of the double bonds had been consumed at 300 min, the resonance at 0.86 ppm continued to grow. Clearly, nucleophilic attack of HA at the β carbons of α,β-unsaturated carbonyls may not be the only side reaction. As the cellulose derivative has many ester linkages, amide-ester exchange is a likely source of side reactions.[30] If the amine attacks the acrylate ester linkage to form the corresponding acrylamide, this new linkage would be retained in the cellulosic product and observed spectroscopically. Primary amine attack at the alkanoate ester linkages (e.g. acetate in CA-Un067) would result in loss of the alkanoate to form a small molecule amide (e.g. *N*-hexyl acetamide in the case of CA-Un067 with hexylamine catalyst), that might or might not be isolated along with the cellulosic product. Another possibility would be retro thiol-Michael addition catalyzed by the amine, followed by aza-Michael addition of HA to the regenerated α,β-unsaturation. However, we feel that this is at most a minor side reaction pathway, as no loss of the thio substituent was observed by proton NMR.

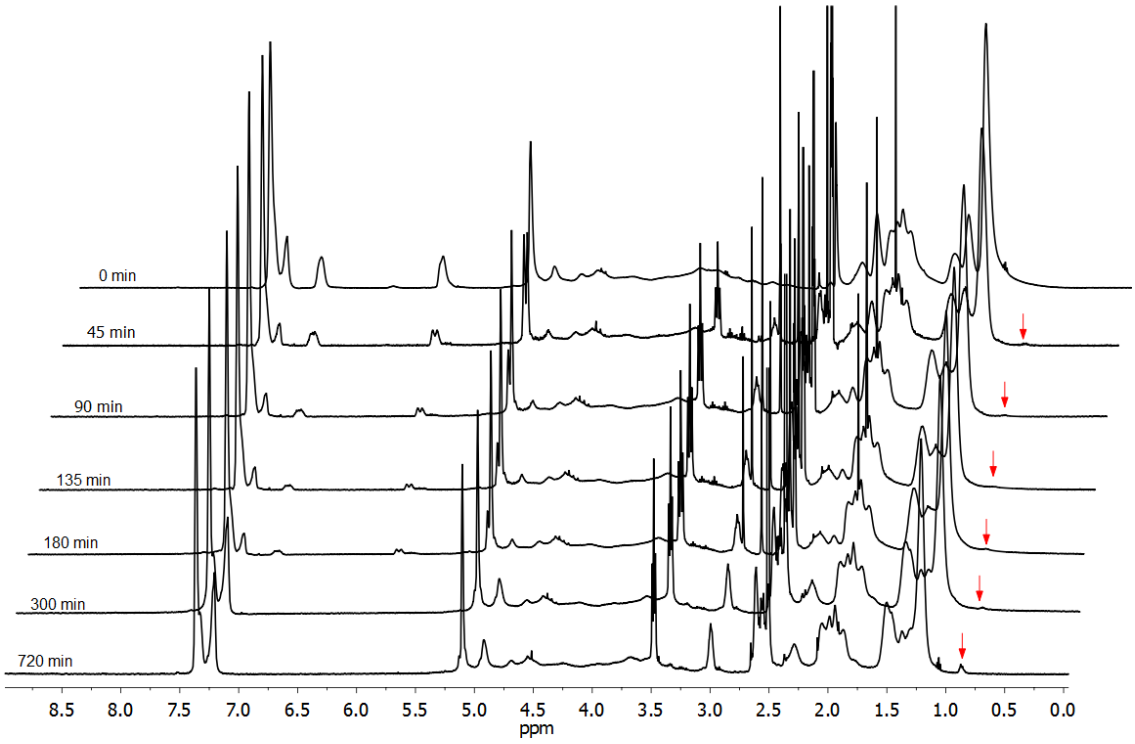


Figure 7.5. ^{1}H NMR spectra for thiol-Michael adducts of 2-ME with CA-Un067-BA under HA catalysis (olefin/2-ME/HA mole ratio equals to 1/2/0.3). Small portions of reaction mixture were withdrawn at each reaction time, precipitated, washed and dried before NMR spectra were recorded.

When TEA was used as the catalyst (**Table 7.2**, polymer **6**, olefin/2-ME/TEA ratio equals to 1/6/6), only 35% olefin conversion was observed, while no by-products were observed (proton NMR, **Fig. S7.5**). This is consistent with observations by previous investigators. Li et al.[29] observed that TEA-catalyzed thiol-Michael reactions proceeded with no noticeable side reactions, while use of primary amine catalysts like HA led to amine addition side products. Chan et al. [31] compared several thiol-Michael catalysts including HA and TEA, and found that TEA catalysis affords a significantly lower rate constant for thiol-Michael addition than does HA. The authors of both studies attributed these observations to the greater nucleophilicity of the primary HA vs. the tertiary TEA amine (while the pK_a values of the two amines are similar (10.75 and 10.56 respectively)) and the resultant difference in catalytic mechanisms.

When 3-MPA was the thiol for 1,4-conjugate addition to CA-Un067-BA (**Table 7.2**, polymer **8** and **9**), we observed results similar to those with 2-ME: HA is a more efficient catalyst than TEA. The carboxylic acid of 3-MPA can form salts with the amine catalyst, and thereby interfere with both base- and nucleophile-catalyzed additions. To overcome these issues, we explored use of a molar excess of amine vs. 3-MPA, intending to overwhelm the deleterious effects of the carboxylic acid functionality. Using TEA catalyst, only 50% of the available double bonds underwent hydrothiolation even when up to 18 eq. of TEA and 9 eq. of 3-MPA were employed, though no side reaction was observed (Fig. S6). Employing HA as catalyst (olefin/3-MPA/HA mole ratio equals to 1/2/3) improved the conversion to a gratifying 87%, as indicated by the amount of residual olefin signal in the proton NMR spectrum (**Figure 7.3**). However, the HA methyl peak at 0.86 ppm was also evident in the ^{1}H NMR spectrum (**Figure 7.3**), and an amide II band around 1566 cm-1 in the FTIR spectrum (**Figure 7.4**) provided further evidence of side reactions involving incorporation of HA into the product.

We also examined the potential of a thiol that also bears amine functionality (cysteamine, CA) as a thiol-Michael partner, due to the intriguing possibility that amine groups could impart to the polymer useful biological activity, for example antibacterial[26] properties. However, in the

systems we investigated using either CA-Un067-BA or other substrates in **Table 7.1**, cross-linked products were obtained. For certain reactions, even if the samples were soluble at the end of the reaction, after dialysis and freeze-drying the afforded products could no longer dissolve even in polar aprotic solvents such as DMSO and DMF. By analogy to the side reactions observed when using HA as catalyst, it is likely that aza-Michael reaction and/or ester-amide exchange are responsible for the observed crosslinking. It should be noted that for substrates that do not possess ester linkages (e.g. CM products that result from reaction of cellulose w-unsaturated ethers with Type II olefin partners other than esters[32,33]) ester-amide interchange cannot be a problem, and therefore crosslinking may not be an issue, since any observed aza-Michael reaction could be circumvented or suppressed[1]. Therefore in these cases it may well be practical and efficient to use amine-bearing thiols like cysteamine as thiol-Michael partners.

Having demonstrated successful conjugate addition of neutral and anionic thiols to the activated olefins of CA-Un067-BA, we wished to explore the scope of the reaction with regard to CM product terminal functionality. We investigated CM products bearing carboxylic acid, hydroxyl, and trimethylammonium functionalities. Not unexpectedly, the acid derivative CA-Un067-AA (**2**) did not react with the thiols under either TEA or HA catalysis. Presumably the negatively charged carboxylate inhibits formation of Michael adducts through either base or nucleophile mechanism due to electrostatic repulsion between the carboxylate and the nearby developing negative charge upon conjugate addition in the position a to the carboxyl. In contrast, and consistent with this explanation, the ester CM products CA-Un067-TMA, CA-Un067-HEA, and CA-Pen079-HEA (**3**, **4**, and **5** in **Table 7.1**) underwent successful thiol-Michael addition reaction with both 2-ME and 3-MPA (**Table 7.3**). HA was an effective catalyst for all of these systems. High conversions (up to 100%) were achieved in most reactions, although minor HA-involved side reactions are observed, especially when reacting with 3-MPA, which requires excess catalyst (**Fig. S7.10** and **S7.12**).

TEA was a more efficient catalyst for these other ester substrates than in reactions with CA-Un067-BA. Differences in nucleophilicity and therefore potentially mechanisms of catalysis of the two amines (HA and TEA) are key factors affecting catalyst efficiency and the extent of side reactions.[31] However, for catalyst efficiency, we believe that other factors such as the ability of the thiol anion to access the b carbon (e.g., steric factors) may also influence reactivity (the electron withdrawing ability of the particular ester group may also influence polymer reactivity

towards conjugate addition, for example by reducing olefin electron density and stabilizing enolate intermediates, but such influences were not clearly important in the current study). CA-Un067-BA has long hydrophobic chains and hydrophobic benzyl rings, which provide a rather hydrophobic milieu for the β carbon. It could be that it is easier for Et_3N or the $Et_3N^+RS^-$ salt to access the b-carbon of the benzoate ester than for the more hydrophilic HA or HA^+RS^- salt. On the other hand, the other CA-based CM product substrate esters, e.g. polymers **3**, **4** and **5**, have hydrophilic ester substituents, making it easier for the more hydrophilic HA or HA^+RS^- salt to access the b carbon. At this point the mechanistic explanation is hypothetical, and more data will be required to refute or confirm this hypothesis. Considering that the use of HA leads to some extent of undesired side reactions, and TEA does not, TEA is a more suitable catalyst for thiol-Michael reactions of polymers **3**, **4** and **5**.

Table 7.3. Thiol-Michael addition of 2-mercaptoethanol and 3-mercaptopropionic acid to different CM products using TEA or HA catalyst.

Cpd.	CM product[a], Polymer #	Thiol (eq.)	Catalyst (eq.)	Time (h)	Conversion[b] (%)	Side reaction[c]
12	CA-Un067-TMA, **3**	2-ME (6)	TEA (6)	12	100	No
13	CA-Un067-TMA, **3**	3-MPA (9)	TEA (18)	12	100	No
14	CA-Un067-HEA, **4**	2-ME (6)	TEA (6)	12	92	No
15	CA-Un067-HEA, **4**	2-ME (3)	HA (2)	5	100	Minimal
16	CA-Un067-HEA, **4**	3-MPA (9)	TEA (18)	12	65	No
17	CA-Un067-HEA, **4**	3-MPA (3)	HA (6)	5	83	Yes
18	CA-Pen079-HEA, **5**	2-ME (6)	TEA (3)	12	> 95	No
19	CA-Pen079-HEA, **5**	3-MPA (5)	TEA (10)	12	> 95	No

[a]The rules for polymer abbreviation are explain in reference 24[24]. [b]Conversion values were calculated by proton NMR (Supporting Information). [c]Extent of side reaction was evaluated qualitatively by proton NMR; quantitative determination not possible due to signal overlap.

Table 7.4 shows the glass transition temperatures and molecular weights of the thiol-Michael products. For certain samples, an endothermic transition at lower temperature was observed, which we attributed to segmental movement of the side chain (b relaxation)[34]. While the transition temperatures differ somewhat from sample to sample, it is difficult to identify a specific structure-property trend. The T_g values are likely affected by multiple factors including side chain length, nature of the terminal functional groups, and specific interactions. Molecular weight data for samples that were charged and/or bore carboxylic acid groups could not be obtained, due to strong interaction between the samples and the SEC column packing. Nevertheless, no significant change in degree of polymerization (DP) was observed according to SEC analysis of the neutral polymers, indicating that the mild thiol-Michael reaction conditions did not cause significant chain scission.

Table 7.4. Physical properties from DSC, SEC analyses of selected samples.

Polymer #	Abbreviation[a]	T_g (β relaxation) (˚C)	M_n (kDa)/DP	Đ[b]
1	CA-Un067-BA	99 (0)	59.6/135	1.7
7	CA-Un067-BA-2ME	84 (1)	76.4/155	2.5
9	CA-Un067-BA-3MPA	111 (11)	NA[c]	NA
3	CA-Un067-TMA	116 (N.O.[d])	NA	NA
12	CA-Un067-TMA-2ME	103 (N.O.)	NA	NA
13	CA-Un067-TMA-3MPA	88 (N.O.)	NA	NA
4	CA-Un067-HEA	87 (10)	50.6/123	1.9
15	CA-Un067-HEA-2ME	108 (10)	49.7/108	2.0
17	CA-Un067-HEA-3MPA	89 (6)	NA	NA
5	CA-Pen079-HEA	125 (N.O.)	52.9/142	1.9
18	CA-Pen079-HEA-2ME	59 (-5)	66.8/154	1.9

19	CA-Pen079-HEA-3MPA	63 (N.O.)	NA	NA

[a]The rules for polymer abbreviation are explain in reference 24[24]. [b]Dispersity. [c]Polymer molecular weight data unavailable due to polymer aggregation and/or polymer-column interaction. [d]Not observed.

7.4 Conclusions

In this study, we present the first examples of elaboration of α,β-unsaturated cellulose esters prepared from CM products by post-CM modification using thiol-Michael addition. The modular characteristics of CM and the "click" nature of thiol-Michael reaction have made both reactions powerful tools in organic and polymer chemistry. The combination of these two powerful reactions for modification of the polysaccharide cellulose in this study creates new methodology for preparation of derivatives of much greater functional diversity, including the option for possessing multiple functional groups, than by using either of these reactions alone. CM reactions initially provide reactive, electron deficient olefins (e.g. α,β-unsaturated esters) that are prone to crosslinking and/or degradation due to abstraction of the labile γ-hydrogens by radical initiators. Therefore these reactive olefins must in any case be eliminated in order to stabilize the CM products for any useful purpose. We show here that these olefins may be excellent substrates for thiol-Michael addition under the right conditions and with the right thiol partner. To be a good substrate for thiol-Michael addition, the olefin should be electron-deficient and the functionality in its vicinity should not interfere with the hydrothiolation process (as does CO_2H, for example). The catalyst plays an important role in thiol-Michael addition. The tertiary amine triethylamine causes no observable side reactions but is sometimes less effective, while the primary amine catalyst n-hexylamine is sometimes more effective, but may promote side reactions by ester-amide exchange or possibly by aza-Michael addition. Amine-containing thiols (e.g. cysteamine) afford crosslinked products with ester CM substrates due to these same side reactions, while other thiol partners showcased in the current study give discrete products with high conversions. Overall, the disadvantage of the reactive conjugated olefin in the initial CM products is thus turned into an advantage, by employing it as the vehicle for introduction of further useful functionality. The double functionalization thus enabled by the combined CM/thiol-Michael addition approach is a strategy that could be of high value for targeting[35], pro-

drug synthesis[36], visualization[37], and any number of other uses. This double modification approach showcased herein also has the potential to be adapted to many other polysaccharides, as well as to even broader areas of polymer synthesis and modification.

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(24) The rules for polymer abbreviation/nomenclature in this manuscript are as follows. For terminally olefinic cellulose esters CA-Un067 and CA-Pen079, CA denotes cellulose acetate DSAc = 1.82; Un denotes undec-10-enoate, and Pen denotes pent-4-enoate, while the numbers 067 and 079 indicate DS of each olefinic side chain. For crossmetathesis products, e.g. CA-Un067-XX, CA-Un067 indicates the starting cellulose ester, while XX stands for the CM partner used in the reaction e.g. AA denotes acrylic acid and BA is benzyl acrylate. For thiol-Michael addition products, e.g. CA-Un067-BA-YY, CA-Un067-BA indicates that the thiol-Michael substrate used is from CM product CA-Un067-BA, while YY stands for the thiol donor employed e.g. 3MPA denotes 3-mercaptopropionic acid.

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Chapter 8: Summary and future work

8.1 Hydroboration-oxidation: A chemoselective route to cellulose ω-hydroxyalkanoate esters

Hydroxy-functionalized cellulose derivatives are of great interest in pharmaceutics, coating and other applications, partially because hydroxyl side chains impart desirable hydrophilicity/hydrophobicity balance and hydrogen bonding to the polymers. They favor polymer-polymer and polymer-small molecule interactions, and water-dispersibility. The synthesis of hydroxy-functionalized cellulose ethers and esters, however, has suffered from imprecise and difficult-to-control chemical structures. Ring opening of epoxides (e.g. ethylene oxide) that has been heavily utilized in cellulose ether synthesis (e.g. 2-hydroxyethyl cellulose) actually affords cellulose-*g*-oligoethers.[1] Similarly, hydroxy-functionalized cellulose esters that have been reported by either direct esterification or ring opening of lactones are cellulose-*g*-polyesters[2] (**Figure 8.1**).

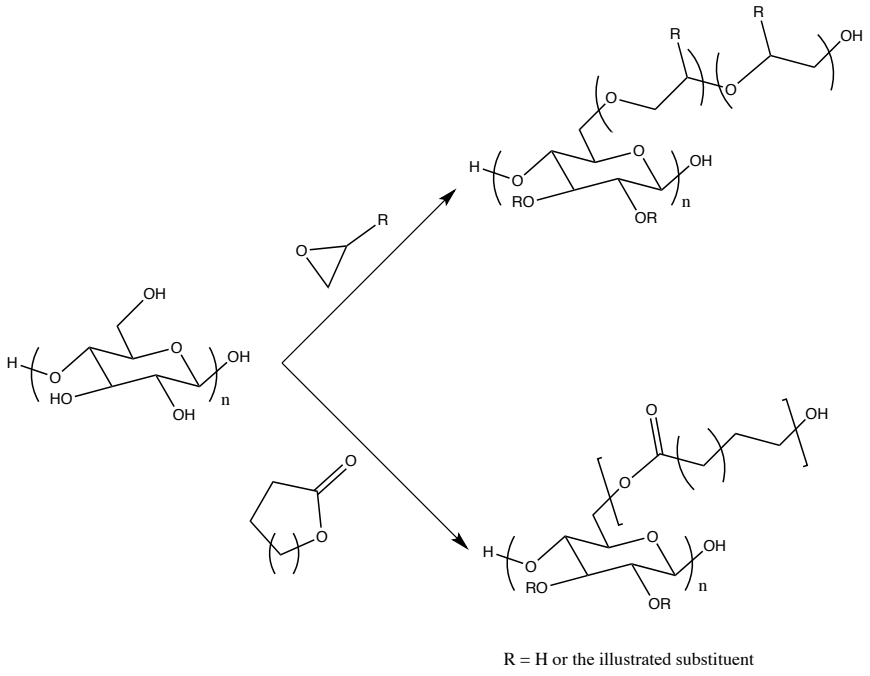


Figure 8.1. Illustration of typical synthesis of hydroxy-functionalized cellulose ethers and esters by ring-opening approaches. (Note that structures are not meant to imply regiospecificity; the particular positions of substitution are shown in all Schemes only for convenience of depiction.)

Chapter 3 of this work explored the viability of utilizing hydroboration-oxidation for synthesis of ω-hydroxyalkanoate cellulose derivatives with well-controlled structures. A previous study by Heinze et al.[3] showed that hydroboration-oxidation of allyl cellulose successfully afforded 3-hydroxypropyl cellulose. To transfer this approach to the synthesis of cellulose esters may have two challenges: a. reduction of ester linkages by hydroboration agents; and b. hydrolysis of ester linkages under the alkaline conditions during oxidation.

Cellulose esters with terminally olefinic side chains (e.g. cellulose acetate undec-10-enoate, CA-Un067) were used as starting materials, which were synthesized from esterification of commercial cellulose esters (e.g. cellulose acetate, CA) with undec-10-enoyl chloride or pent-4-enoyl chloride. The starting materials were then modified through hydroboration and oxidation reactions, as described in **Figure 8.2**.

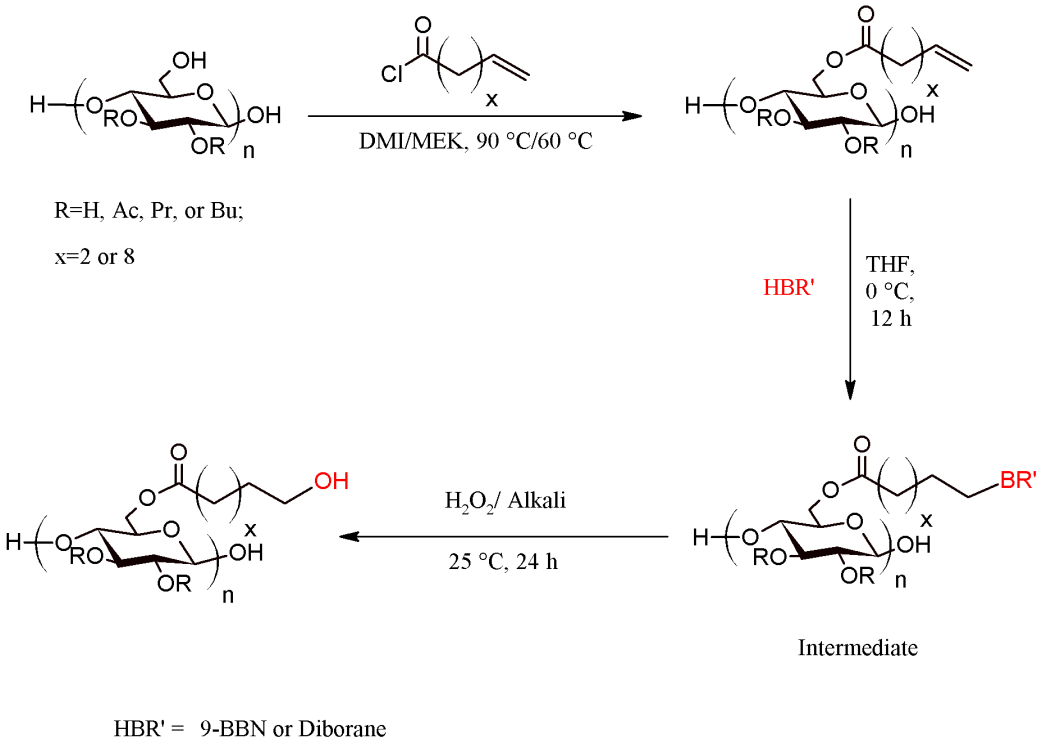


Figure 8.2. General scheme for the synthesis of hydroxy-substituted cellulose esters by hydroboration-oxidation reaction.

Results in **Chapter 3** showed that 9-BBN is a better hydroboration reagent compared with diborane. Under otherwise identical conditions, use of diborane as hydroboration reagent

caused obvious loss of ester substituents, while no observable change of DS of esters was observed when 9-BBN was employed. The results also showed that the alkaline condition (using NaOH as the alkali during oxidation) used in the synthesis of hydroxy-functionalized cellulose ethers was not applicable in current research, as the ester linkages were susceptible to rapid alkaline saponification. To this end, sodium hydroxide was substituted by the much weaker base sodium acetate during oxidation, and the ester hydrolysis issue was successfully circumvented. Overall, synthetic pathways to cellulose ω-hydroxyalkanoates were successfully established, which include a. esterification of cellulose to generate cellulose esters with terminally olefinic side chains, b. hydroboration by 9-BBN to get organoborane intermediates, and c. oxidation of the borane intermediates in the presence of a mild base such as sodium acetate.

The cellulose esters bearing primary alcohols prepared by the new hydroboration-oxidation sequence will be of great interest in ASD formulation studies.[4] This synthetic approach, with the cross-metathesis approach that will be discussed in the following chapters, provides cellulose derivatives with identical structures except for functional groups at the side chain terminus. Thus, they provide an excellent platform for structure-property relationship study. Even so, it should be noted that all the derivatives synthesized in this study are still hydrophobic to a large extent due to long hydrocarbon side chain length and low DS of hydroxyl. This hydrophobicity may limit the effectiveness of the polymers in ASD formulation, as well as its usefulness in other areas. So in future work, exploring the reaction using polysaccharide starting materials with higher intrinsic solubility, shorter terminally unsaturated side chains, and/or possessing other, more hydrophilic groups may be of interest.

8.2 Olefin cross-metathesis as a source of polysaccharide derivatives: Cellulose ω-carboxyalkanoates

Olefin metathesis has opened up new areas in polymer chemistry by providing new polymerization methods and enabling new polymeric architectures. The previous applications of olefin metathesis in polysaccharide modification had been discouraging, as such efforts often led to crosslinked products due to dominant and uncontrolled self-metathesis reaction[5]. The study in **Chapter 4** provides a brand new approach in polysaccharide functionalization by olefin cross-metathesis. As shown in **Figure 8.3**, cellulose esters with terminal olefin side chains, prepared from esterification of commercial cellulose esters with undec-10-enoyl chloride, were employed

as starting materials. It is noteworthy that the same family of cellulose esters was used in hydroboration-oxidation, which permits ready structure-property relationship comparison of products from the two chemistries. Cellulose esters with pendant terminal olefins were then reacted with an excess of acrylic acid under the catalysis of Hoveyda-Grubbs 2nd generation catalyst. The CM reaction then afforded a carboxylic acid functionalized cellulose derivative along with ethylene by-product under mild conditions. The α,β-unsaturation generated from CM was responsible for crosslinking during storage through a free radical mechanism (likely by abstraction of γ-H and radical recombination). This crosslinking was suppressed by adding free radical scavengers such as BHT.

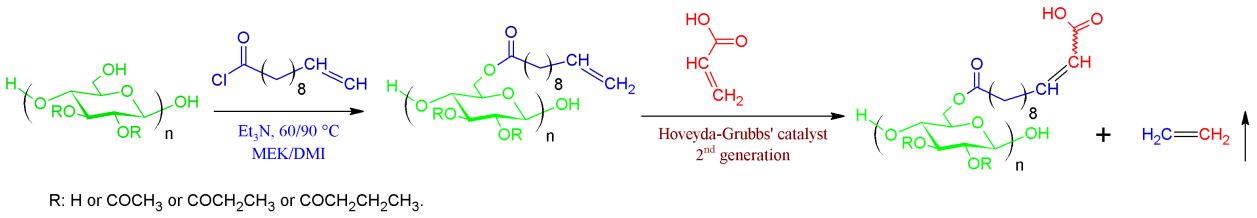


Figure 8.3. General two-step synthetic method for cellulose ω-carboxyalkanoate derivatives.

This approach has opened a new door for the synthesis of carboxylic acid functionalized cellulose esters. Comparing with previous synthetic methods[6,7], the current sequence has provided a more rapid, efficient and simpler approach. The follow-up work and more in-depth study of this reaction including exploration of a broader spectrum of CM partners and the elimination of α,β-unsaturation was discussed in the following chapters.

8.3 Olefin cross-metathesis, a mild, modular approach to functionalized cellulose esters

Conventionally, the chemistry chosen to prepare a functionalized cellulose derivative highly depends on the functional group that one wants to append on the cellulose backbone. For example, a protection-deprotection strategy was selected to prepare cellulose ω-carboxyalkanoates[6], while hydroboration-oxidation was employed when primary alcohol was the desired terminal functionality[8]. The proof-of-concept work in **Chapter 4** has showed the power of CM for synthesis of cellulose esters with carboxylic acid pendant groups. In **Chapter 5**, we extend the scope of this reaction to another family of CM partners, acrylates, and thus expand the ability of this chemistry to produce broader spectrum of functionalized cellulose esters.

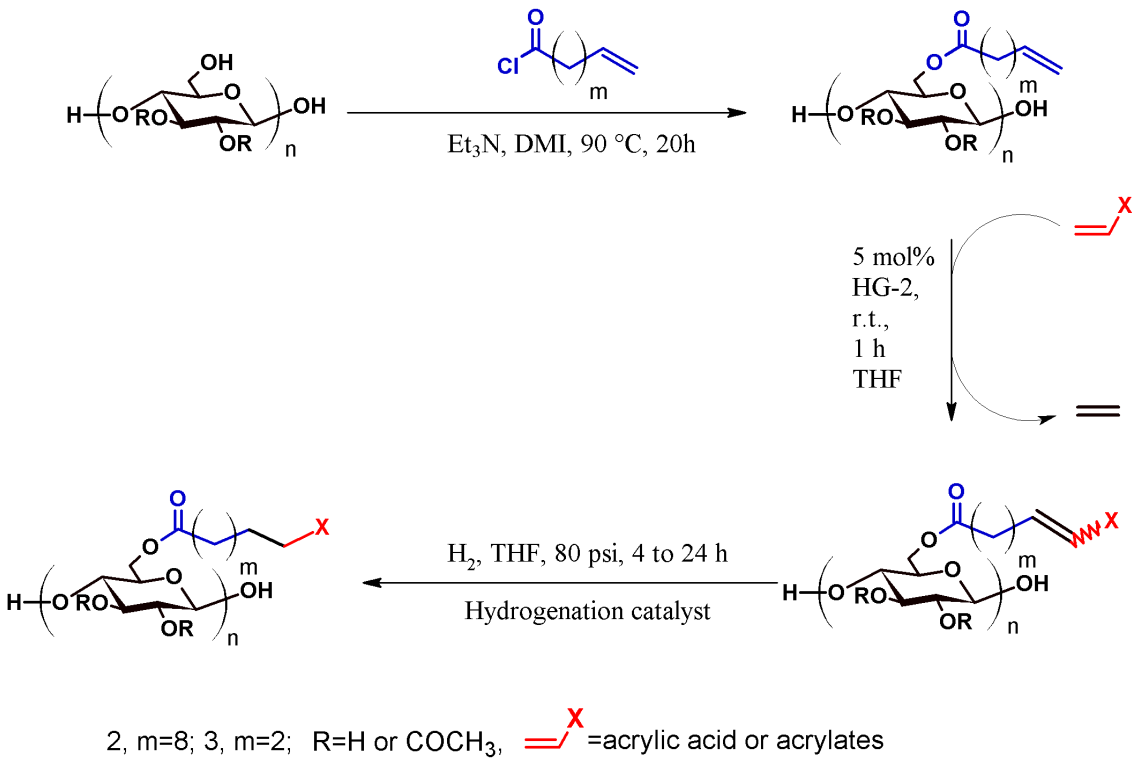
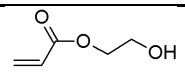
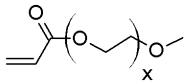
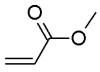
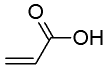
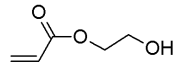
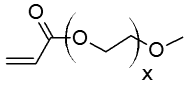


Figure 8.4. General three-step synthetic method for functionalized cellulose esters via olefin cross-metathesis.

In this study, commercial cellulose acetate (CA-320S, M_n ~ 38.0 kDa, DP ~ 151, DS(Ac) ~ 1.82 (data provided by supplier)) were reacted with pent-4-enoyl chloride or undec-10-enoyl chloride to get cellulose pentenoate ($DS_{pentenoate}$ = 0.56, CA-Pen056) and cellulose undecenoate ($DS_{undecenoates}$ = 0.67, CA-Un067) derivatives. CM between the afforded terminally olefinic cellulose esters and a variety of acrylate CM partners, including methyl acrylate, 2-hydroxyethyl acrylate, and poly(ethyl glycol) methyl ether acrylate was performed in THF (**Table 8.1**). Complete conversions to CM products were achieved within 1 h at ambient temperature.

Table 8.1. CM of olefin terminated cellulose acetate with acrylic acid and acrylates.

Starting cellulose ester	CM partner (abbr.)	E/Z ratio[a]	Conversion[a]	Yield %	DS (X)[a]	DS (Ac)[a]
CA-Un067	-	-	-	93	0.67	1.88
CA-Un067	 (AA)	16.7	~100%	93	0.77	1.77

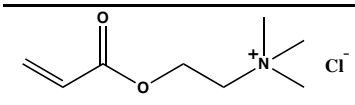
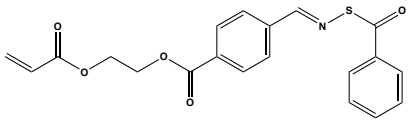
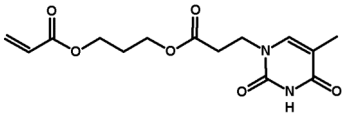
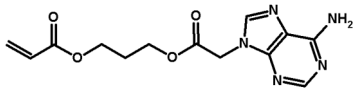
CA-Un067	 (HEA)	15.2	~100%	97	0.65	1.78
CA-Un067	 (PEGMEA)	33.3	~100%	84	NA[b]	NA[b]
CA-Un067	 (MA)	16.7	~100%	94	0.67	NA[b]
CA-Pen056	-	-	-	89	0.56	1.80
CA-Pen056	 (AA)	15.5	~100%	94	0.56	1.85
CA-Pen056	 (HEA)	9.7	~100%	96	0.56	1.86
CA-Pen056	 (PEGMEA)	8.3	~100%	88	NA[b]	NA[b]

[a]*X* represents the functional group pending on cellulose backbone by CM; DS values were determined by proton NMR. [b]DS values not available due to peak overlapping.

In the previous chapter, we have observed crosslinking of CM products which we believe was due to the α,β-unsaturation generated after CM. In **Chapter 5**, we propose several different hydrogenation pathways to eliminate the α,β-unsaturation. Heterogeneous hydrogenation catalyzed by Pd/C worked well for cellulose esters with long side chains (CA-Un067 as the starting material), and was able to completely reduce the double bonds under 80 psi H_2 for 12 h in either a one pot CM-hydrogenation reaction or a separate hydrogenation. For cellulose esters with shorter side chain (CA-Pen056 as the starting material), homogeneous catalysts (Wilkinson's or Crabtree's catalyst) were more efficient to completely eliminate the double bonds. Under heterogeneous catalysis, olefins tethered to the cellulose backbone by short chains may not gain conformations that are favorable for the occurrence of hydrogenation. Although hydrogenation was successful, it should be noted that increased DP and partial loss of solubility

were observed for polymers from homogeneous hydrogenation. We attribute this to undetermined side reactions or physical crosslinking by the metal catalysts.

Table 8.2. Chemically and structurally more complex CM partners and the corresponding CM reactions.

CM partner[a]	Functional group	Solvent	Catalyst loading	Terminal olefin/CM partner ratio	Conversion
	Trimethyl ammonium	Acetic acid	5 mol%	1:20	100%
	S-aroylthiooxime	THF	3 mol%	1:20	100%
	Thymine	Acetic acid	6 mol%	1:20	> 90%
	Adenine	Acetic acid	6 mol%	1:20	~ 40%

[a]The aroylthiooxime CM partner was kindly provided by Prof. John Matson and Jeffrey Foster; the thymine and adenine CM partner were kindly provided by Prof. Timothy Long and Keren Zhang.

We show in this chapter a new chemistry in polysaccharide modification that is mild, efficient, and modular. These features are of great value for studies that investigate the effect of different functional groups on certain polymer properties. Moreover, besides the CM partners listed in **Chapter 5**, we have also examined some chemically and structurally more complex molecules as CM partners (**Table 8.2**). Comparing with simple acrylates, these molecules will impart more complex functionality to cellulose derivatives. For example, S-aroylthiooxime

groups are H_2S donors and release H_2S by triggers like cysteine.[9] Nucleobase-functionalized polymers may exhibit morphological behaviors such as self-assembly and phase-separation.[10] With these preliminary results, we have all the reason to expect that CM in polysaccharide functionalization will enable the conjugation of chemically complex and sensitive compounds. From this perspective, the current research opens up possibilities not only in the design and structure-property relationship study of ASD polymers, but also in other demanding applications. Based on the preliminary research shown in **Table 8.2**, it will be of interest to further investigate potential properties of the functionalized cellulose esters such as H_2S releasing profile, molecular recognition, antibacterial, and antifouling properties. Further study in conjugating other molecules such as fluorophores and drug molecules may also be interesting.

8.4 Synthesis of amide-functionalized cellulose esters by olefin cross-metathesis

Amide-functionalized polymers present exciting possibilities in ASD polymer design due to polymer-polymer and polymer-small molecule interactions through the hydrogen bonding capability provided by the amide functionality.[11] More than that, such H-bonding interactions enable a wide range of interesting polymer properties and applications such as molecular recognition and strengthened mechanical performance.

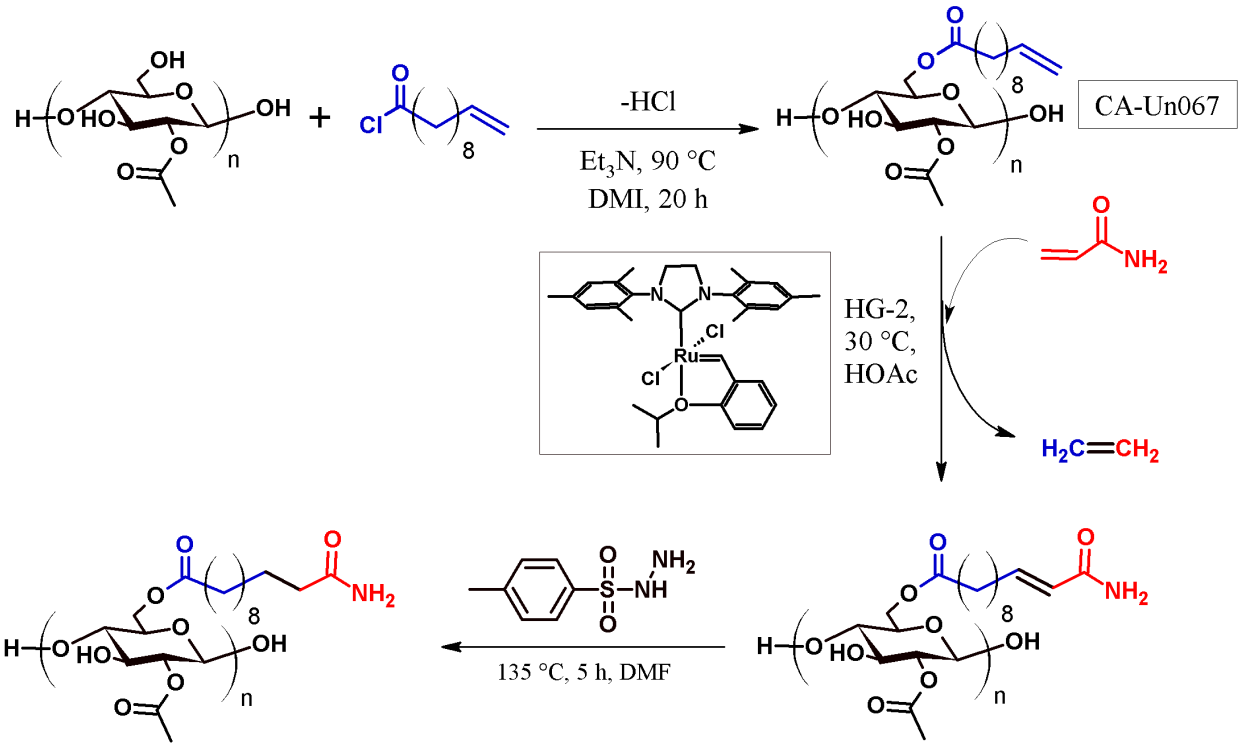


Figure 8.5. General three-step synthetic method for amide-functionalized cellulose esters via olefin cross-metathesis.

Previous research in cellulose functionalization provided only very limited capability to append amide groups onto cellulose. Our study of using CM in cellulose modification had focused on CM partners such as acrylic acid and various acrylate esters. The research described in **Chapter 6** established a new synthetic pathway towards amide-functionalized cellulose esters via olefin CM reaction between terminally olefinic cellulose esters and acrylamide CM partners under the catalysis of Hoveyda-Grubbs 2nd generation catalyst, followed by hydrogenation to reduce the α,β-unsaturation to afford stable final products (**Figure 8.5**). One of the challenges in this reaction sequence was that the conversions of CM reactions involving acrylamide as CM partner were usually low in typical olefin metathesis solvents (e.g. THF and DCM, 50% conversion was achieved using up to 12 mol% of the catalyst in THF). We hypothesized that the amide nitrogen might chelate with ruthenium catalysts (**Figure 8.6**) and suppress its turnover rate, which may result in low conversions. We then proposed the use of an organic acid such as acetic acid as solvent for this reaction, which significantly improved the conversion to over 99%. This significant jump in conversion was attributed to the fact that acetic acid can interact with the amide through H-bonding and weaken the chelation between the amide and the metal catalyst. Based on the same hypothesis, we proposed that electron density of the amide double bond as well as the chelation ability of nitrogen, which are related to solvent-amide interaction and electron-withdrawing/donating effect and steric hindrance of substituents on amides, play important roles in the conversion of the CM reaction. We believe this interpretation will provide a generally useful guideline for improving the conversion of acrylamide related CM reactions.

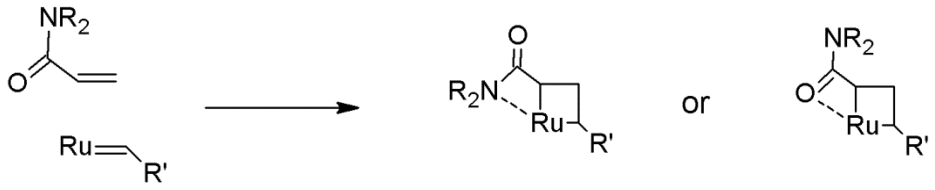


Figure 8.6. Proposed amide chelation of the catalyst in CM reactions with acrylamides.

We also found a more general method to reduce the α,β-unsaturation of the amide CM products by transfer hydrogenation using *p*-toluenesulfonyl hydrazide, which generates diimide via thermolysis and thereby reduces the double bond (**Figure 8.7**). Although we have developed

different hydrogenation strategies including using heterogeneous Pd/C or homogeneous Wilkinson's or Crabtree's catalyst, these methods are sometimes not compatible with all the CM products due to either solubility or reactivity issues leading to side reactions. The current diimide method has proven successful for most of the CM products of cellulose esters that have been discussed in this dissertation, regardless the side chain length and functionality.

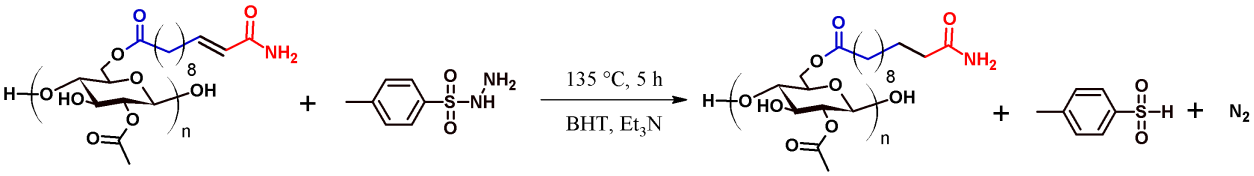


Figure 8.7. Transfer hydrogenation of CA-Un067-Aam using *p*-toluenesulfonyl hydrazide.

Moreover, it is interesting that unlike most other cellulose derivatives with long side chains, the glass transition temperatures of which decreased after CM functionalization, the amide-functionalized cellulose esters exhibited higher glass transition temperatures after CM. We believe that this is very likely due to the hydrogen bonding capability of the introduced amide group, which restricts the segmental motion of cellulosic chain and increases transition temperatures. To better learn the potential impact of amide functionality, it will be worthwhile to perform further characterization of the amide-functionalized cellulose derivatives. For example, DMA will give further confirmation of the glass transition temperature, and will also indicate the impact absorbing potential of the materials by analyzing the area under the tanδ curve.[12]

8.5 Multifunctional cellulose esters by olefin cross-metathesis and thiol-Michael addition

In **Chapter 7**, we showcased for the first time a double modification strategy towards multifunctional cellulose esters. **Figure 8.8** illustrates the concept of the double modification strategy. In a CM reaction between terminally olefinic cellulose esters and a CM partner (e.g. acrylate), α,β-unsaturation was generated, which could be used as a handle for a subsequent thiol-Michael addition reaction. In such a way, the cellulose derivative would acquire both X functional group from CM and Y functional group from thiol-Michael addition.

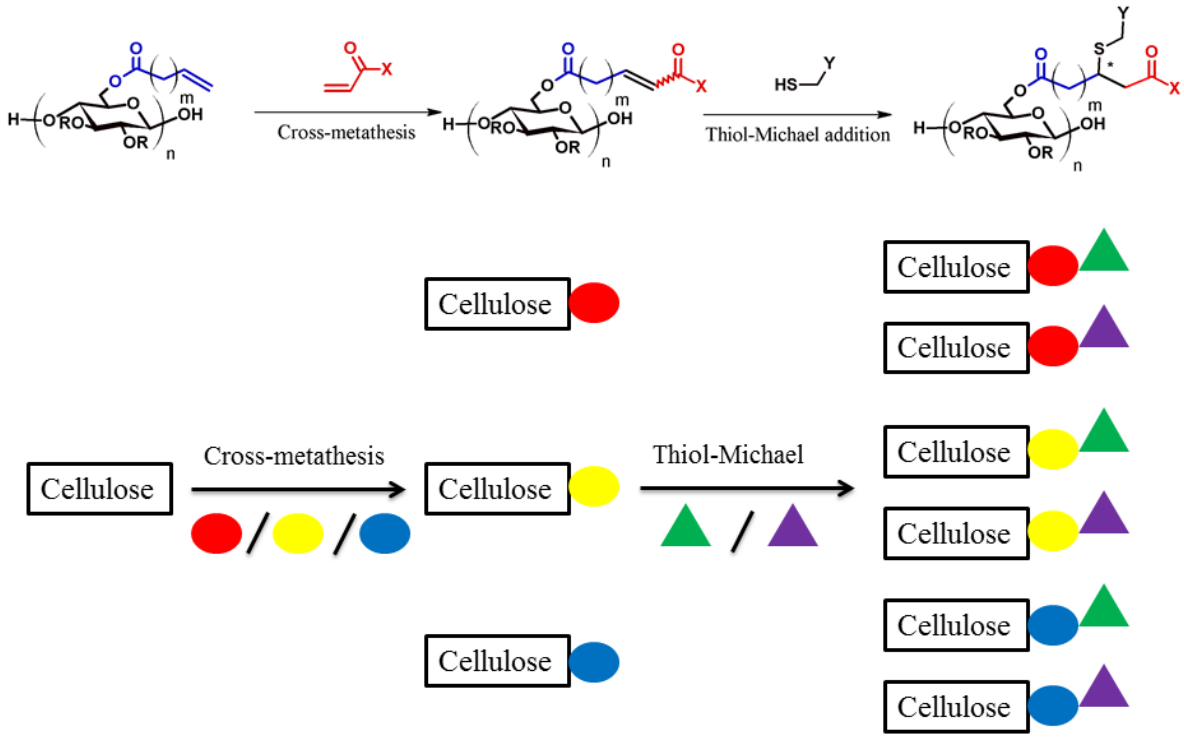


Figure 8.8. Schematic illustration of olefin cross-metathesis and thiol-Michael addition towards functionalized cellulose derivatives. The circles of different colors represent different functionalities introduced by CM, and the triangles of different colors represent different functionalities introduced by thiol-Michael addition.

In this research, CM products with different functional groups such as benzyl ester, hydroxyl, trimethylammonium, and carboxylic acid were chosen as substrates for thiol-Michael addition to test the compatibility of the reaction. 2-Mercaptoethanol, 3-mercaptopropionic acid and cysteamine were selected as thiols with representative functionalities. The thiol-Michael reaction was conducted in DMSO at ambient temperature using hexylamine or triethylamine as catalyst. It was found that CM products with α,β-unsaturated carboxylic acid could interfere with the hydrothiolation process due to electrostatic repulsion between thiol anion and carboxylate anion during the reaction. Cysteamine as a thiol led to crosslinked products due to possible side reactions (e.g. ester-amide exchange and aza-Michael addition). Other than these exceptions, the

post-CM modification approach showed very good applicability to various polymer-thiol combinations.

It is also worthy to note that although hexylamine as a catalyst seemed to be more efficient than triethylamine, evidence of side reactions (likely ester-amide exchange and aza-Michael addition by the primary amine) was observed. Therefore one task in future work will be to identify a catalyst which on the one hand is more efficient, and on the other avoids side reactions. A sterically hindered amine (e.g. 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU))[13,14] may be a better choice as catalyst.

Through our previous study[15-18], CM has proven a modular and click-like pathway in cellulose functionalization. The proof-of-concept work in the current research demonstrates that the combination of the modular CM reaction with the thiol-Michael click reaction creates a new methodology for preparation of derivatives with much more diverse functionalities and structural architectures than by using either of the reactions alone. More than that, this methodology opens up a broad range of opportunities for preparation of cellulose derivatives with interesting chemical and physical properties and potential applications. From this perspective, future work will focus more on characterization of physical properties of these polymers and purposefully designing polymers for specific applications. For example, positively charged (polymer **3** in **Chapter 7**) and zwitterionic polymers (polymer **13** in **Chapter 7**) may of interest as antibacterial, anti-fouling and pH-responsive polymers. Thus, taking the advantages of these reactions, a series of polymers with various side chain length and functionality combinations can be prepared. Physical properties like contact angle, polymer absorption and aggregation behavior under different pH, and antibacterial and anti-fouling functions of the polymers can be examined and compared.

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Appendix: Supplementary Figures and Tables

<u>Chapter 3:</u> Hydroboration-oxidation: A chemoselective route to cellulose ω-hydroxyalkanoate esters

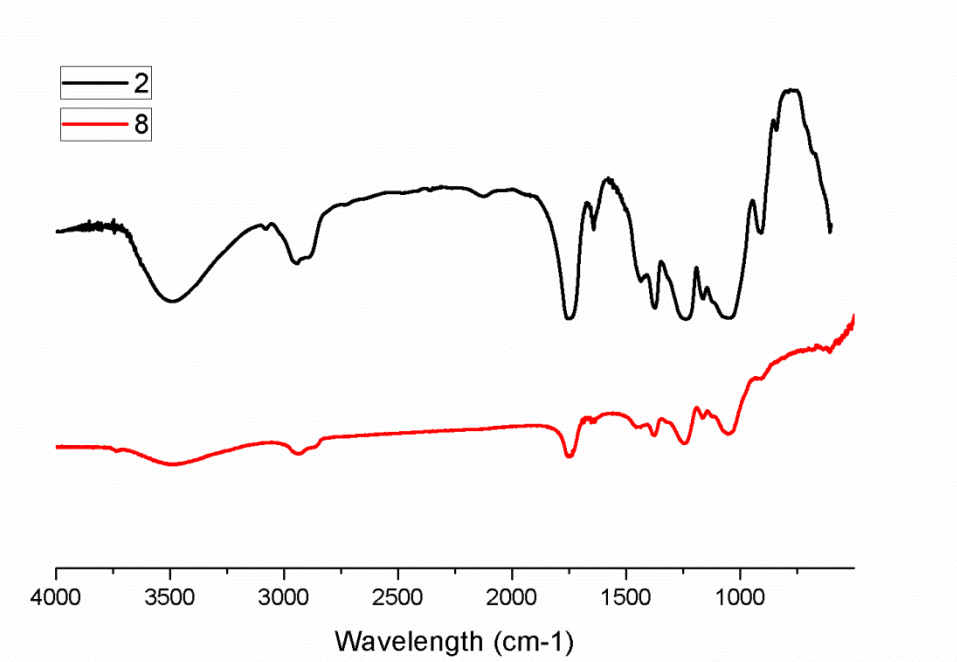


Fig. S3.1. FTIR spectra of CA-Pen056 (**2**) and product Hb-CA-Pen056 (**8**).

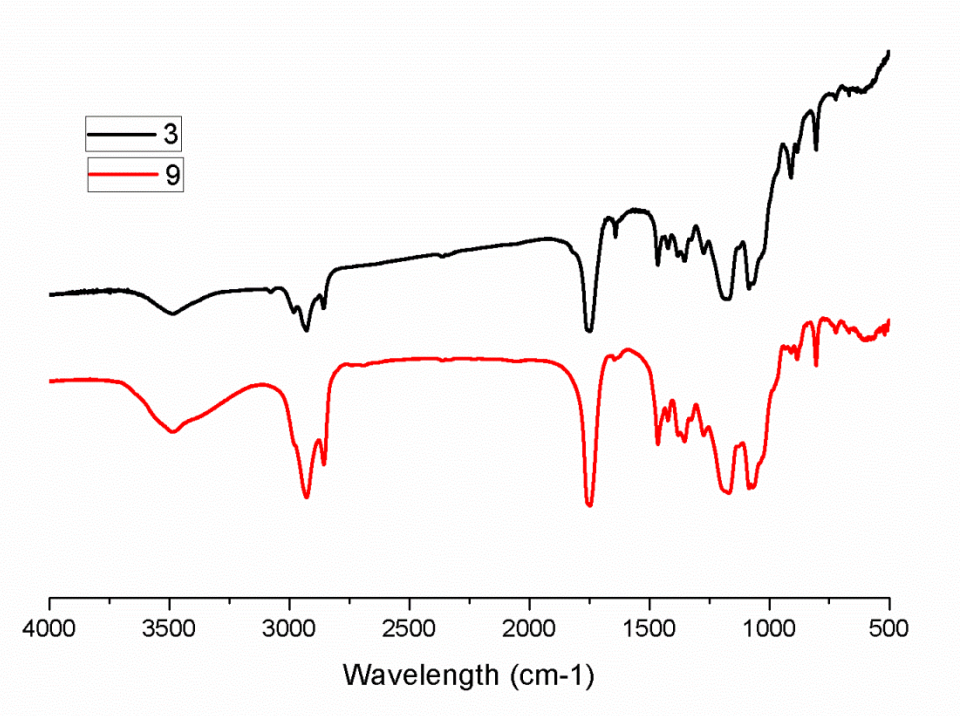


Fig. S3.2. FTIR spectra of CAP-Un057 (**3**) and product Hb-CAP-Un057 (**9**).

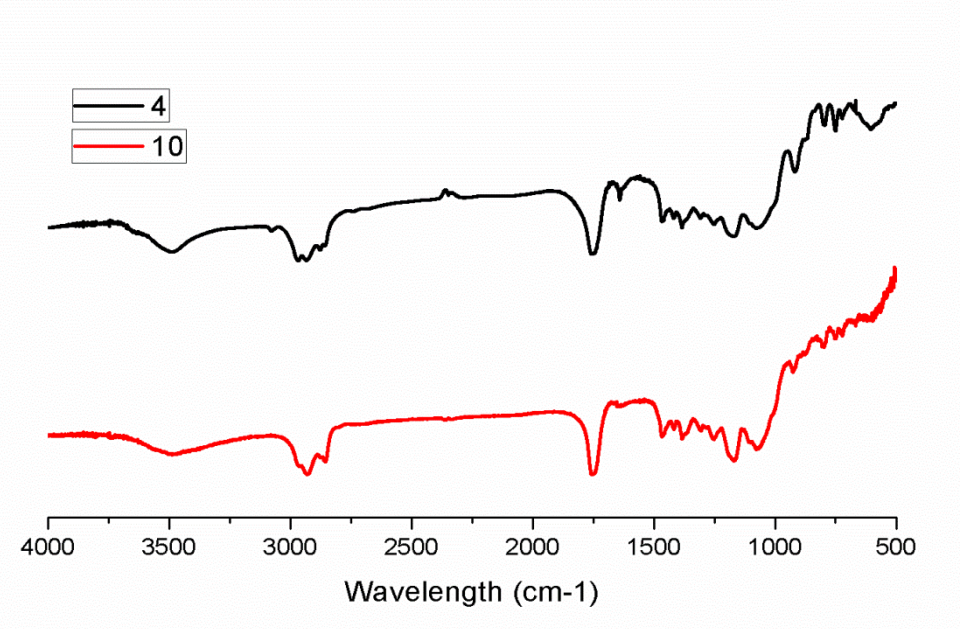


Fig. S3.3. FTIR spectra of CAB-Un059 (**4**) and product Hb-CAB-Un059 (**10**).

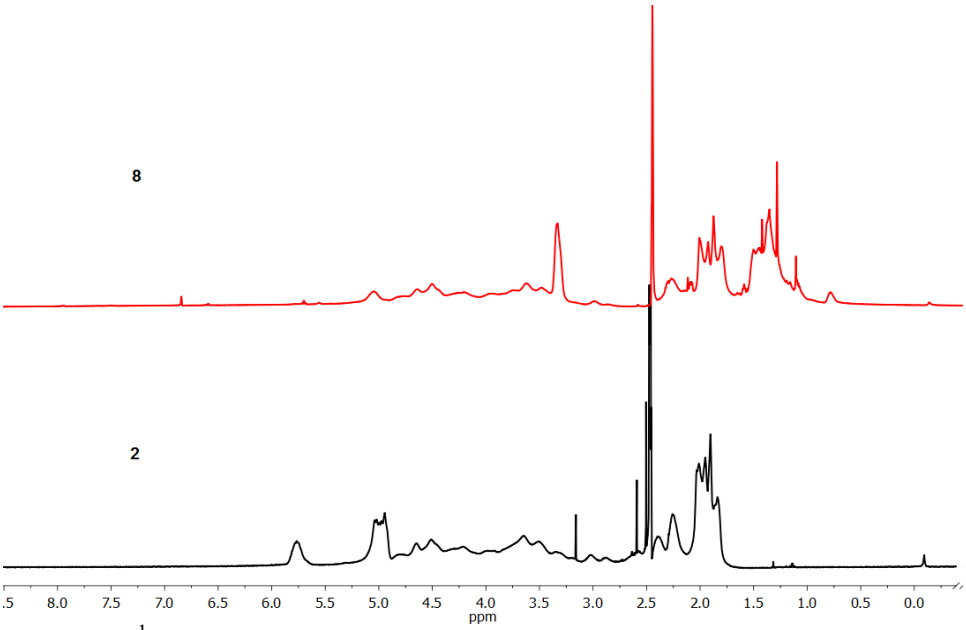


Fig. S3.4. ^{1}H NMR spectra of CA-Pen056 (**2**) and product Hb-CA-Pen056 (**8**).

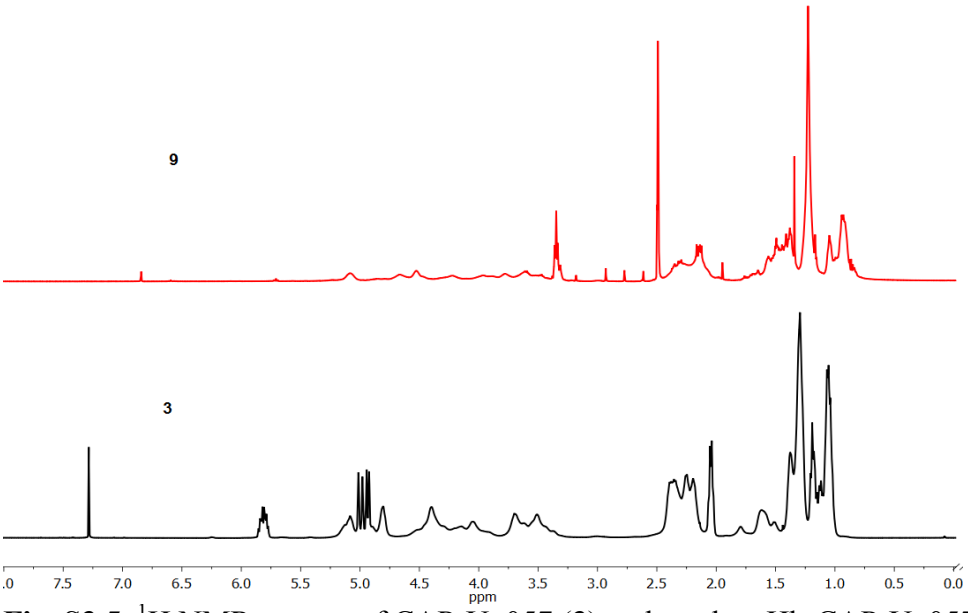


Fig. S3.5. ^{1}H NMR spectra of CAP-Un057 (**3**) and product Hb-CAP-Un057 (**9**).

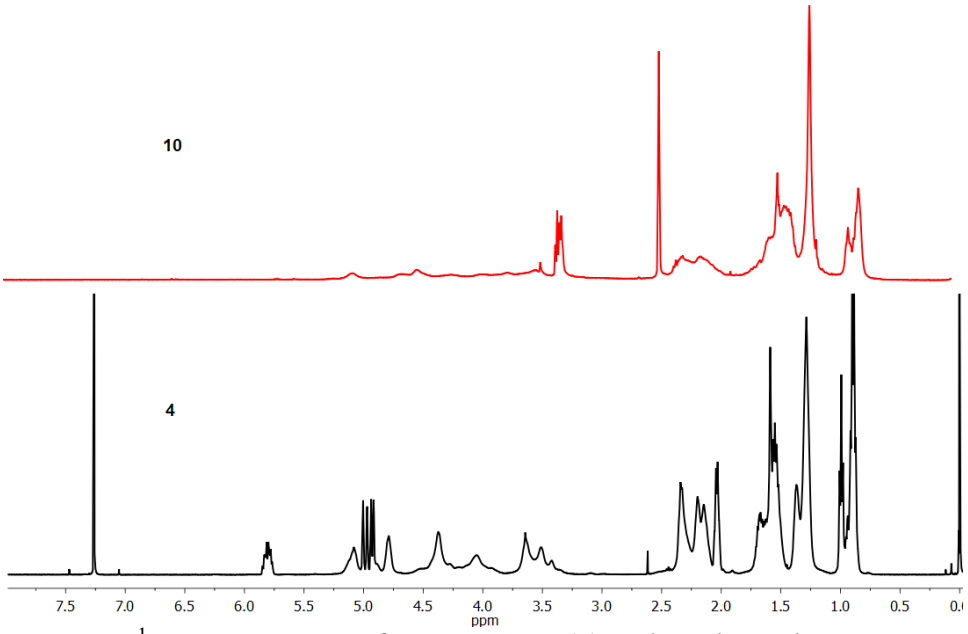


Fig. S3.6. ^{1}H NMR spectra of CAB-Un059 (**4**) and product Hb-CAB-Un059 (**10**).

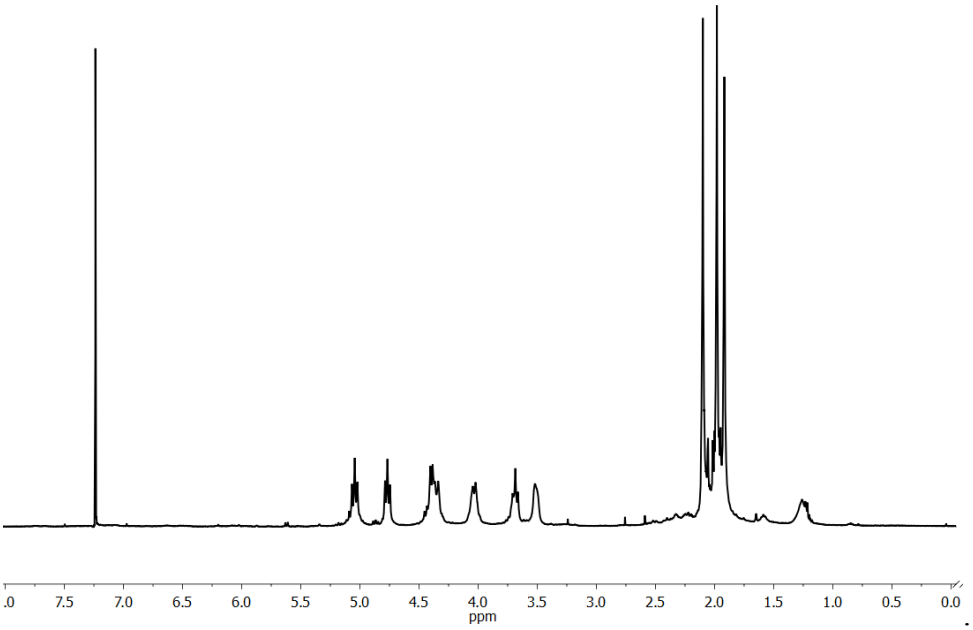
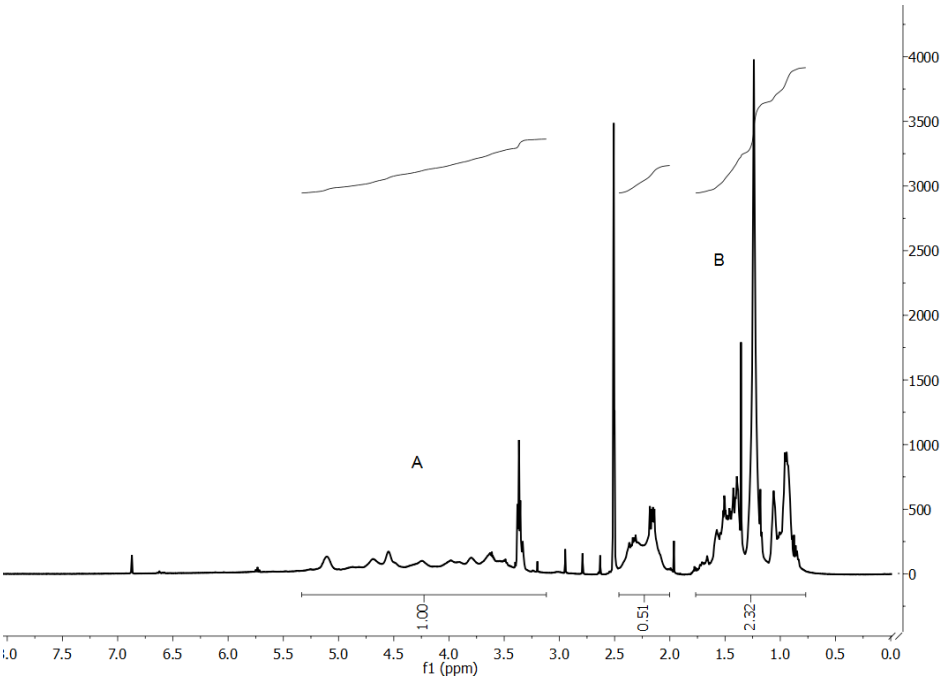


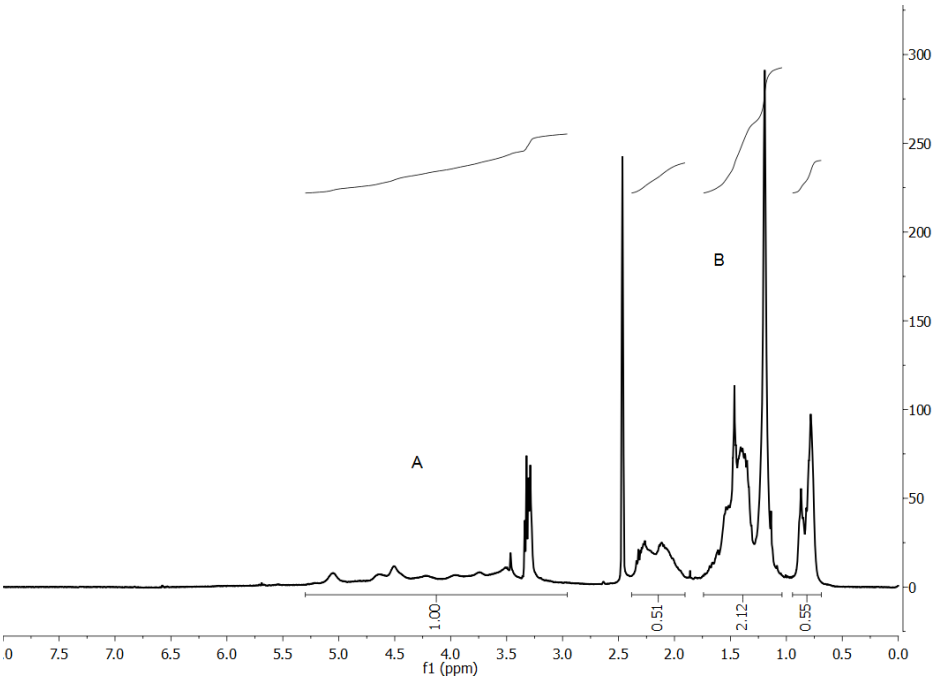
Fig. S3.7. [1]H NMR spectrum of peracetylated product of **5**.



Theoretical value of $\frac{I(A)}{I(B)} = \frac{2.09\times5+0.04\times3+0.57\times18}{7+2\times0.57} = 2.70$ (The actual ratio would be lower if any ester substituents lost)

Actual value of $\frac{I(A)}{I(B)} = \frac{0.51+2.32}{1.00} = 2.83$ (The higher value may be due to the 9-BBN residue signal in the B region)

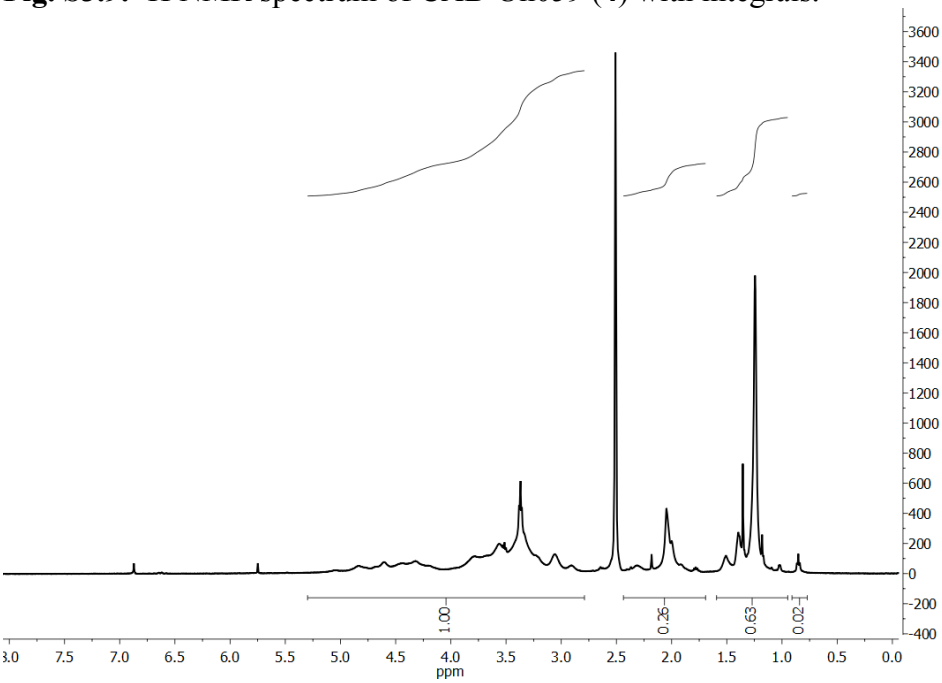
Fig. S3.8. [1]H NMR spectrum of CAP-Un057 (**3**) with integrals.



Theoretical value of $\frac{I(A)}{I(B)} = \frac{1.99\times7+0.14\times3+0.59\times18}{7+2\times0.59} = 3.19$

Actual value of $\frac{I(A)}{I(B)} = \frac{0.51+2.12+0.55}{1.00} = 3.18$

Fig. S3.9. ^{1}H NMR spectrum of CAB-Un059 (**4**) with integrals.



Theoretical value of $\frac{I(A)}{I(B)} = \frac{1.82\times3+0.67\times18}{7+2\times0.67} = 2.10$

Actual value of $\frac{I(A)}{I(B)} = \frac{0.26+0.63+0.02}{1.00} = 0.91$

Fig. S3.10. ^{1}H NMR spectrum of product **7** with integrals.

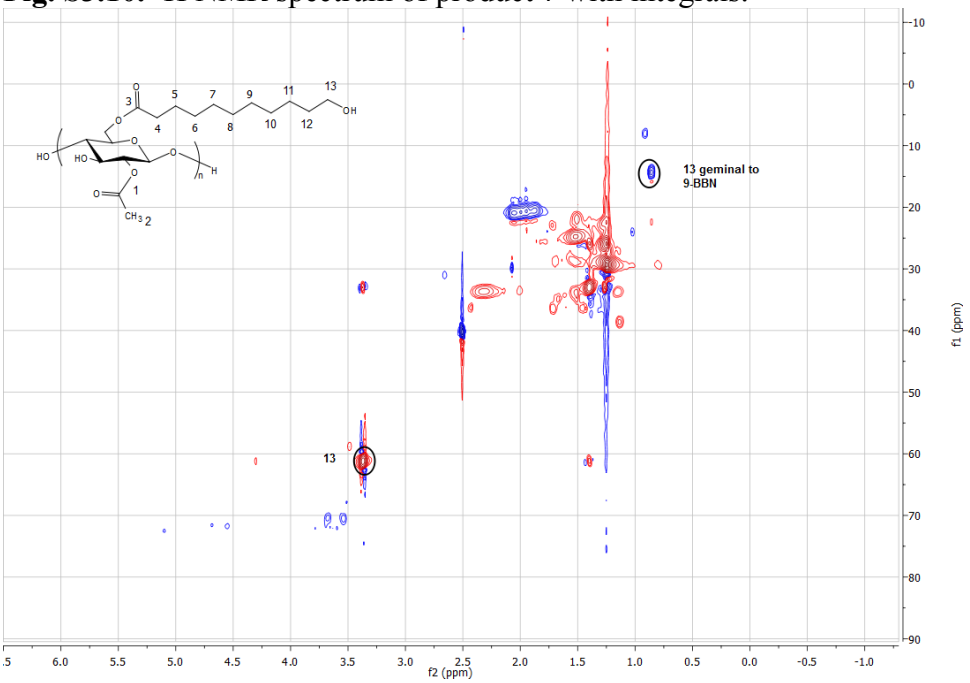


Fig. S3.11. ^{1}H-^{13}C HSQC spectrum of product Hb-CA-Un067.

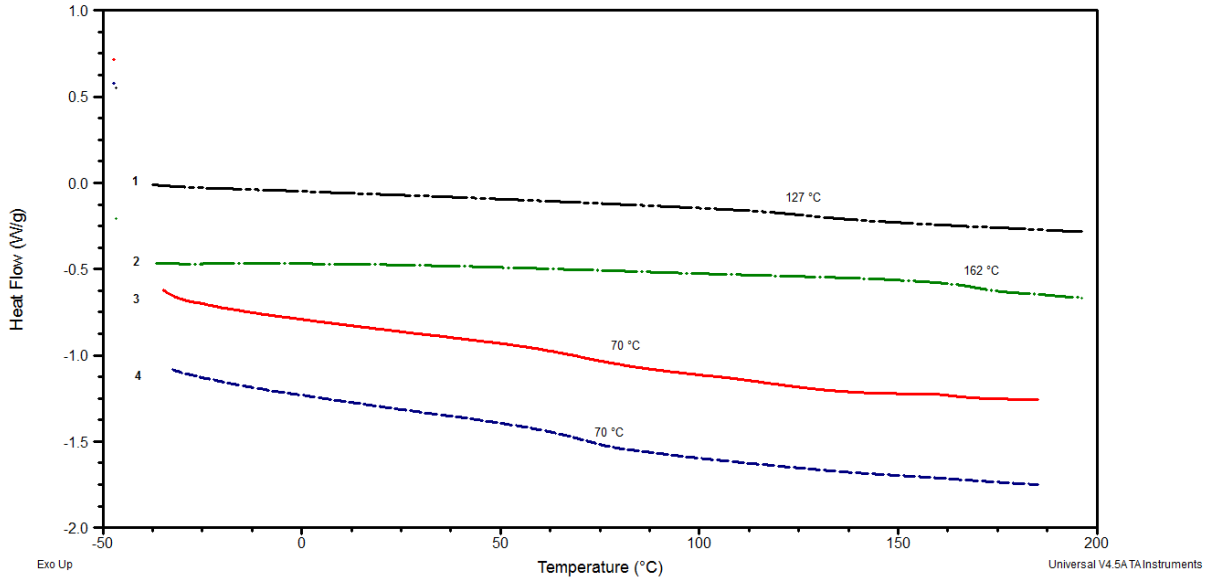


Fig. S3.12. DSC data of the terminally olefinic cellulose esters CA-Un067 (**1**), CA-Pen056 (**2**), CAP-Un057 (**3**) and CAB-Un059 (**4**).

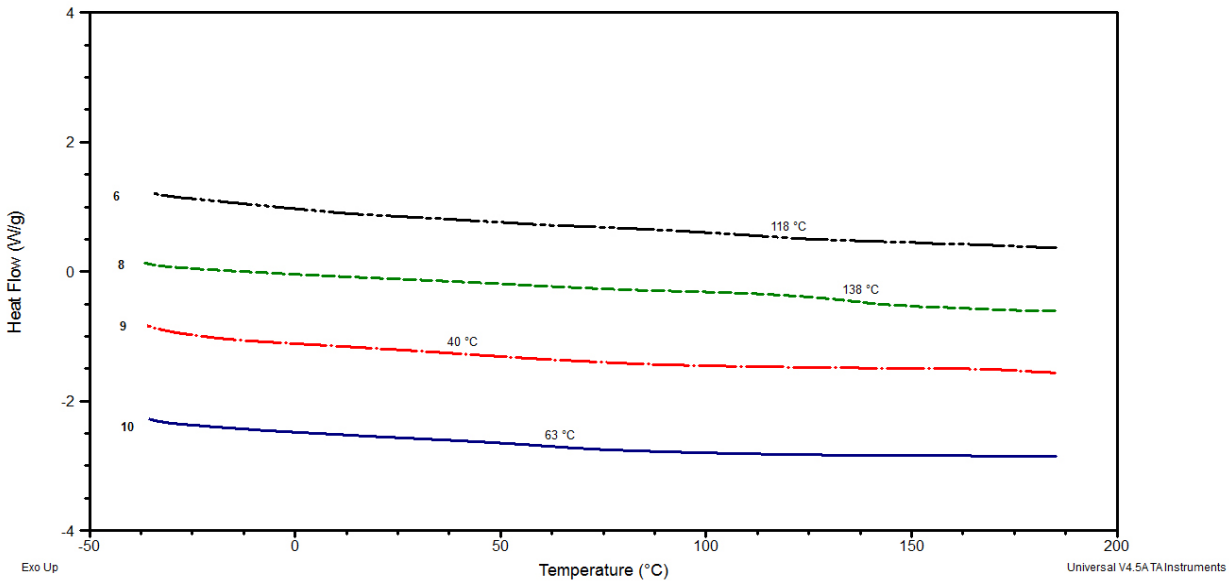


Fig. S3.13. DSC data of the hydroboration-oxidation products Hb-CA-Un067 (**6**), Hb-CA-Pen056 (**8**), Hb-CAP-Un057 (**9**) and Hb-CAB-Un059 (**10**).

Table. S3.1. pH values of reaction media in the oxidation reaction with 3.4 mL of 3M NaOAc, or H_2O, or 3M NaOH.[a]

	Measurement 1	Measurement 2	Measurement 3	Average
NaOAc	5.48	5.47	5.47	5.47
H_2O	3.12	3.12	3.12	3.12
NaOH	6.76	6.77	6.76	6.76

[a]pH was measured using a pH meter during reaction. Measurement 1, 2 and 3 were recorded at 1, 6, and 12 h after the addition of the "base".

Chapter 4: Olefin cross-metathesis as a source of polysaccharide derivatives: Cellulose ω-carboxyalkanoates

We include here FTIR, ^{1}H NMR, ^{1}H-^{13}C HSQC, and DSC spectra of new compounds prepared that we did not include in the body of the manuscript. We also included the predicted proton NMR spectra of dodec-2-endioic acid and dimerized dodec-2-endioic acid via a free radical mechanism, which were compared to the real spectra of our hydrolyzed samples. All the spectra, unless otherwise stated below, were acquired using instruments and methods as described in the Experimental Section of the paper.

FTIR Spectra

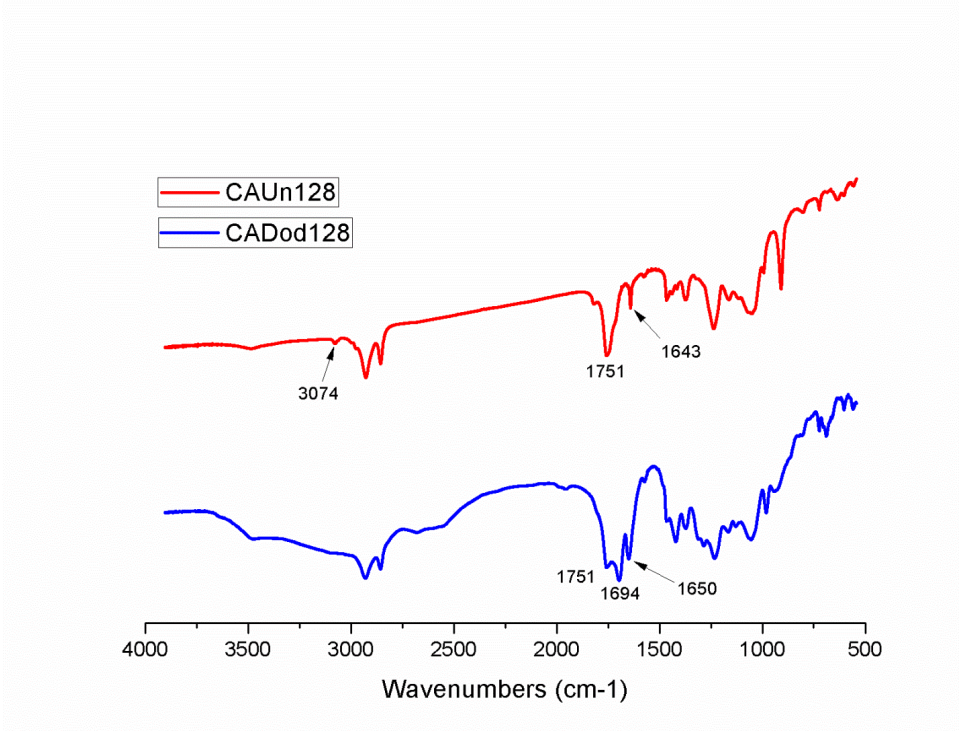


Fig. S4.1. FTIR spectra of CAUn128 and CADod128.

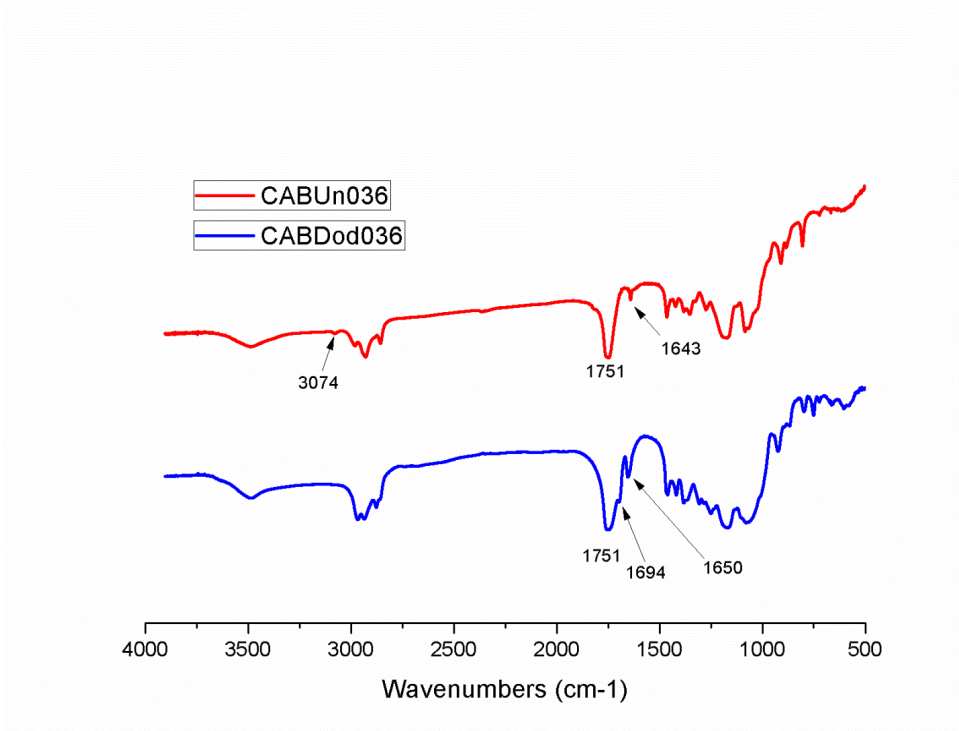


Fig. S4.2. FTIR spectra of CABUn036 and CABDod036.

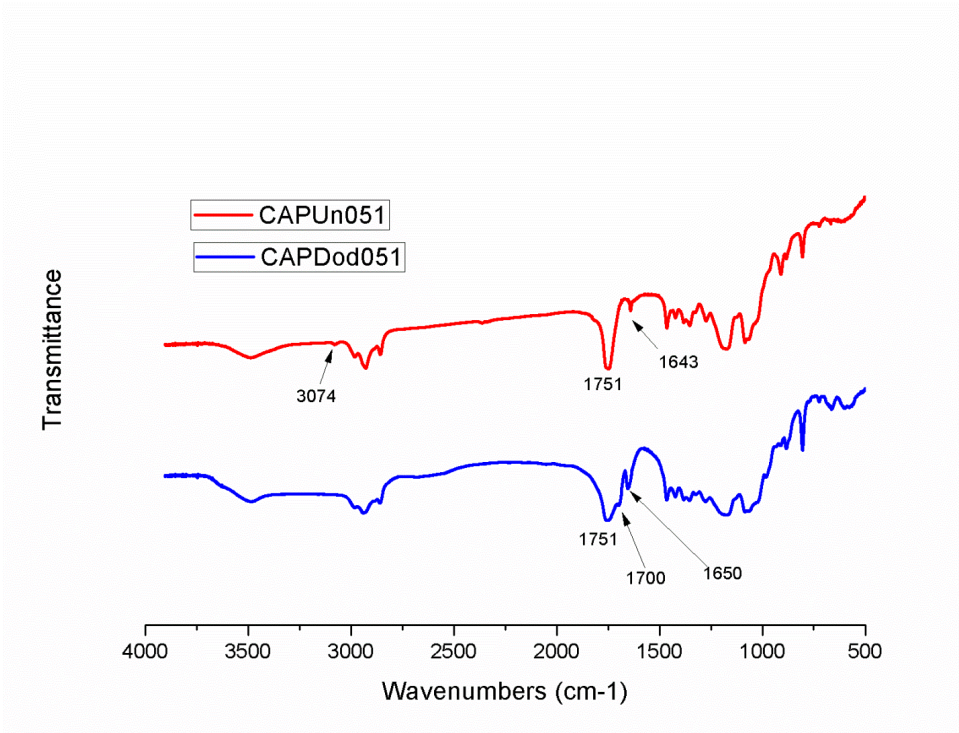
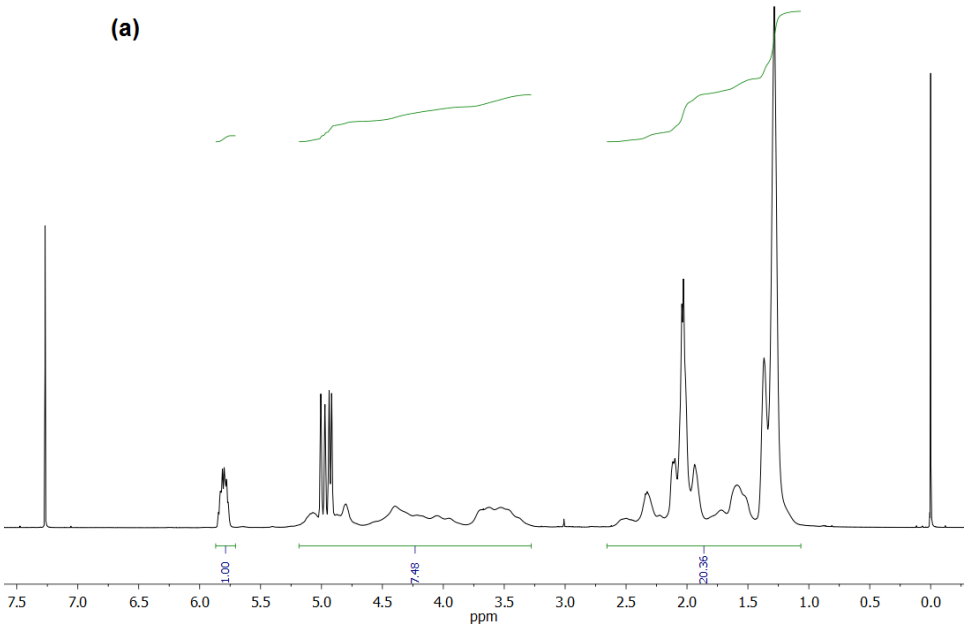


Fig. S3.3. FTIR spectra of CAPUn051 and CAPDod051.

NMR Spectra

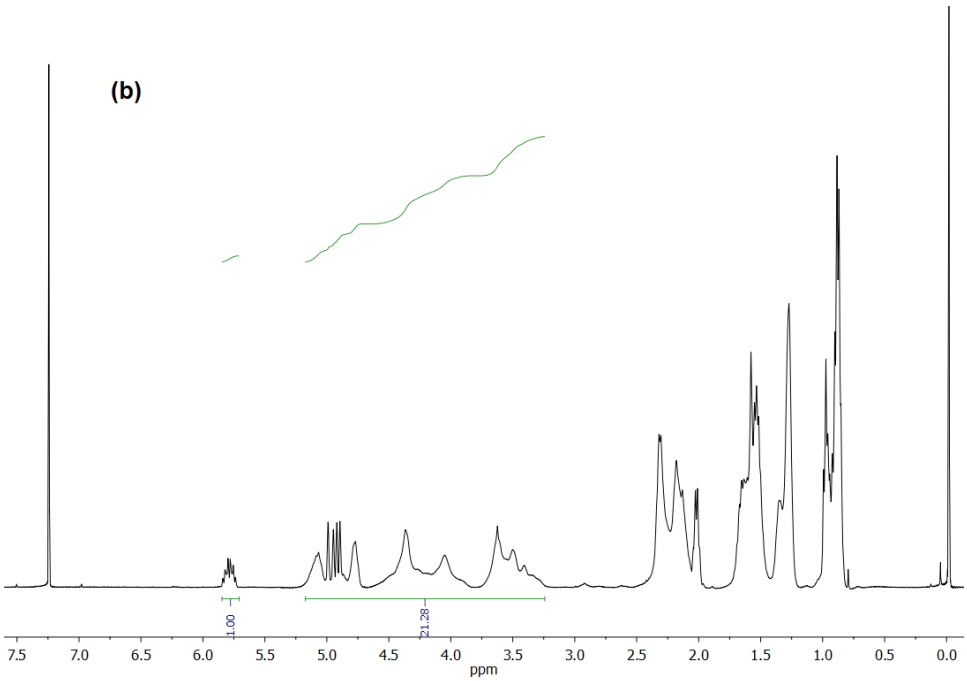


DS (Un) was calculated according to the equation:

$$\frac{7 + 2[DS(Un)]}{DS(Un)} = \frac{7.48}{1.00}$$

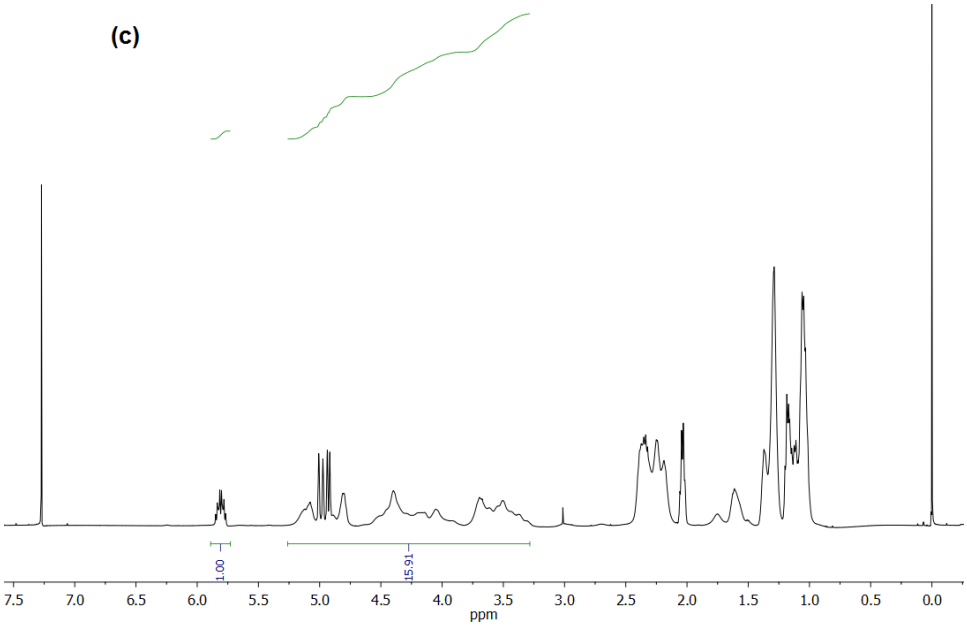
DS (Ac) was calculated according to the equation:

$$\frac{8{\times}2{\times}DS(Un) + 3{\times}DS(Ac)}{DS(Un)} = \frac{20.36}{1.00}$$



DS (Un) was calculated according to the equation:

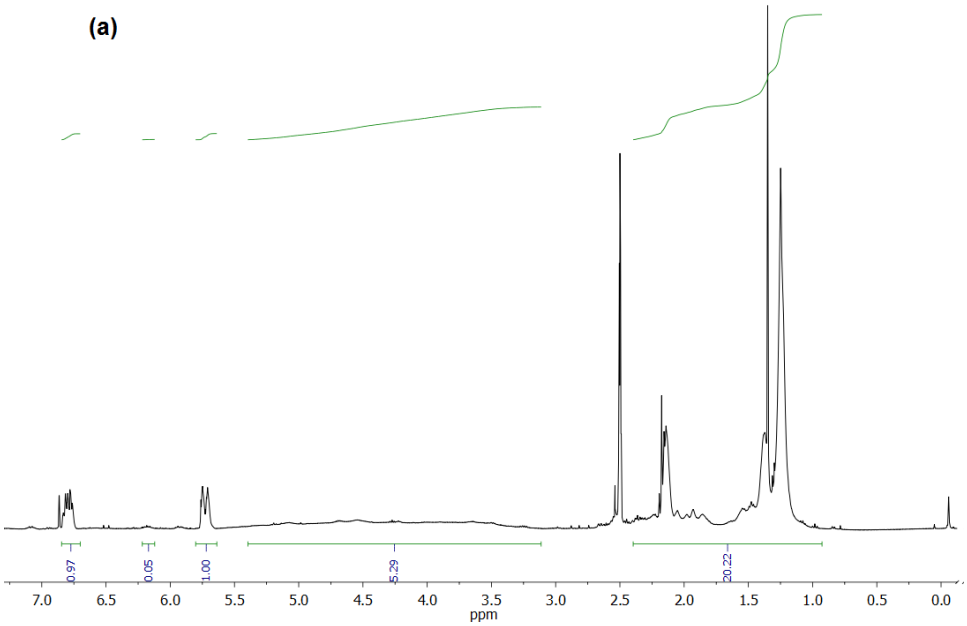
$$\frac{7 + 2[DS(Un)]}{DS(Un)} = \frac{21.28}{1.00}$$



DS (Un) was calculated according to the equation:

$$\frac{7 + 2[DS(Un)]}{DS(Un)} = \frac{15.91}{1.00}$$

Fig. S4.4. [1]H-NMR spectra of CAUn128 (a), CABUn036 (b), and CAPUn051 (c).



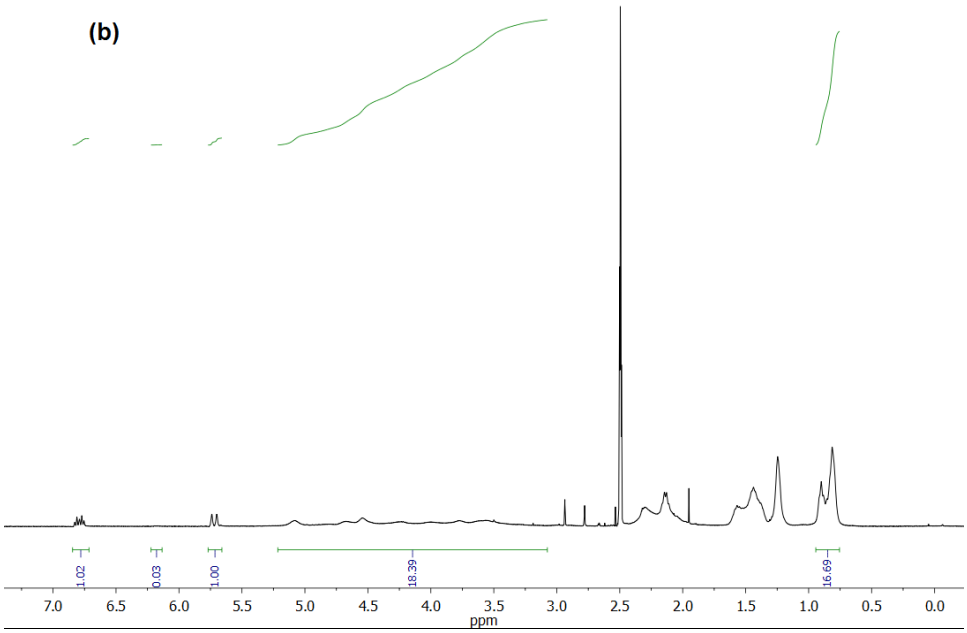
DS (Dod) was calculated according to the equation:

$$\frac{7 + 2[DS(Dod)]}{DS(Dod)} = \frac{5.29}{1.00}$$

DS (Ac) was calculated according to the equation:

$$\frac{8\times2\times DS(Un) + 3\times DS(Ac)}{DS(Un)} = \frac{20.22}{1.00}$$

(Here and in the following calculation, we use corresponding DS (Un) determined in S4 instead of DS (Dod) because the former is more accurate)

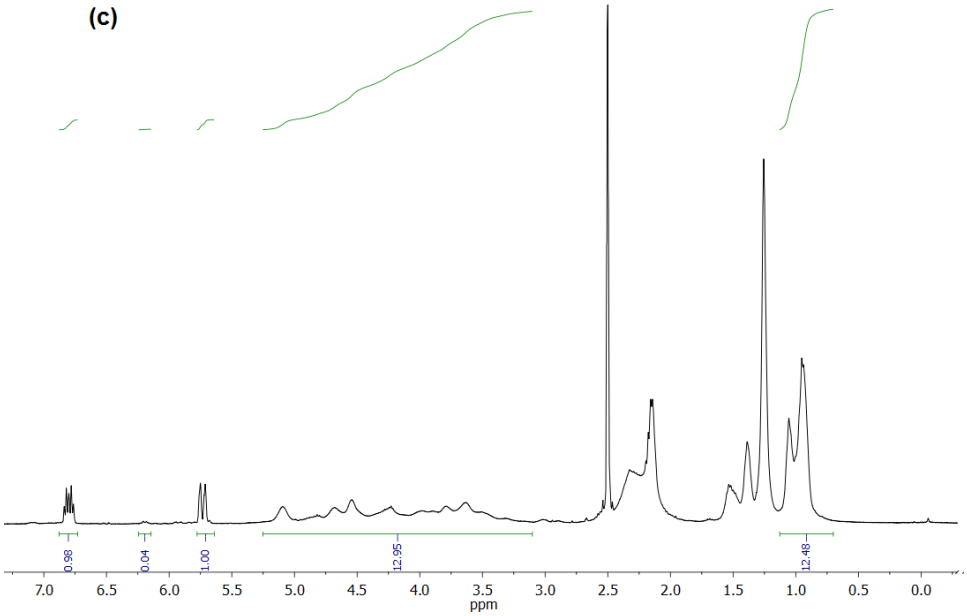


DS (Dod) was calculated according to the equation:

$$\frac{7 + 2[DS(Dod)]}{DS(Dod)} = \frac{18.39}{1.00}$$

DS (Bu) was calculated according to the equation:

$$\frac{3 \times DS(Bu)}{DS(Un)} = \frac{16.69}{1.00}$$



DS (Dod) was calculated according to the equation:

$$\frac{7 + 2[DS(Dod)]}{DS(Dod)} = \frac{12.95}{1.00}$$

DS (Pr) was calculated according to the equation:

$$\frac{3 \times DS(Pr)}{DS(Un)} = \frac{12.48}{1.00}$$

Fig. S4.5. ^{1}H-NMR spectra of CADod128 (a), CABDod036 (b), and CAPDod051 (c).



Fig. S4.6. ^{1}H-^{13}C HSQC spectrum of CADod067.

DSC Data

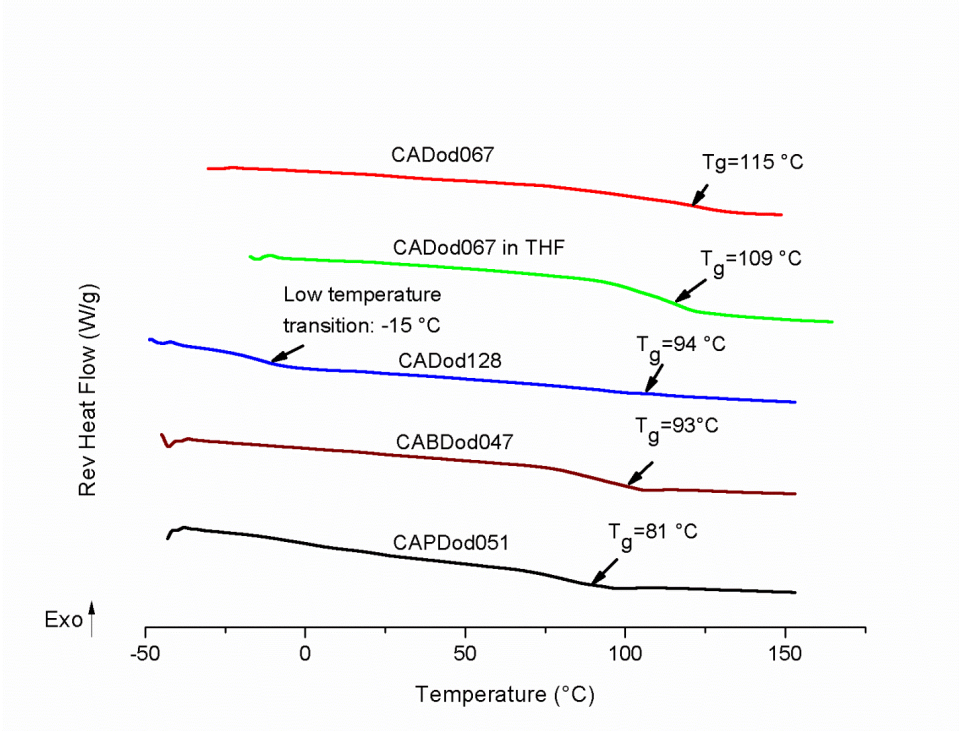


Fig. S4.7. DSC Analysis of CADod067, CADod067 in THF, CADod128, CABDod036 and CAPDod051.

Predicted NMR Spectra

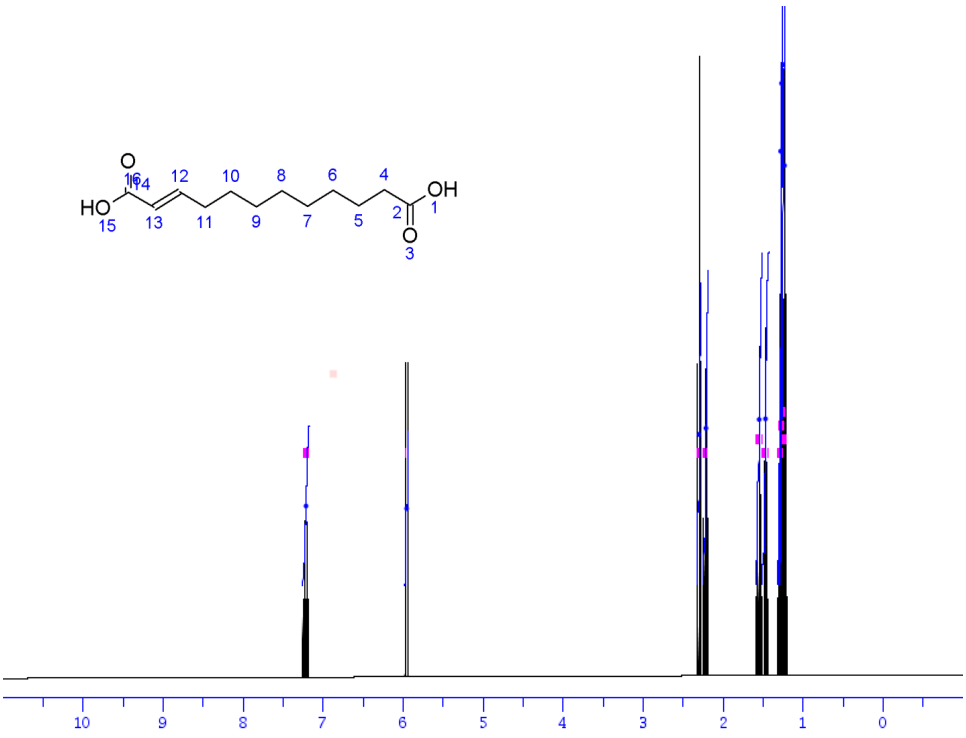


Fig. S4.8. Predicted ^{1}H-NMR spectrum of dodec-2-endioic acid. 7.213 (12, 1H, dt, J=15.755, J=6.803), 5.954 (13, 1H, d, J=15.755), 2.213 (11, 2H, td, J=7.433, J=6.803), 1.469 (10, 2H, tt, J=7.681, J=7.433), 1.231-1.279 (6, 7, 8, 9, 8H, m), 1.549 (5, 2H, tt, J=7.667, J=7.367), 2.299 (4, 2H, t, J=7.367) (Note: the spectrum was predicted in the website http://www.nmrdb.org/predictor).

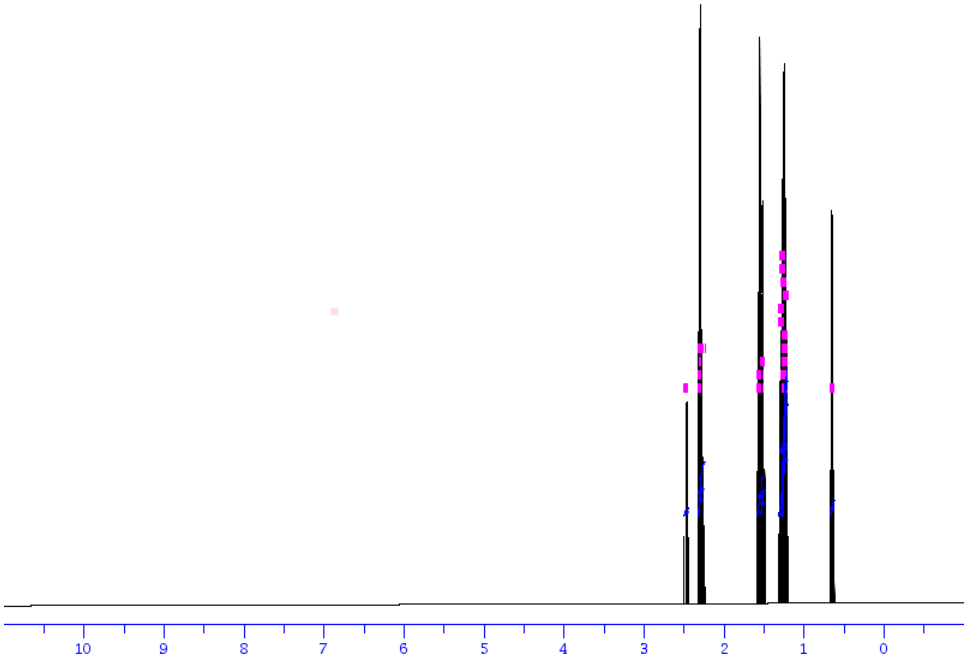


Fig. S4.9. Predicted ^{1}H-NMR spectrum of dimerized dodec-2-endioic acid. 0.648 (6, 2H, dt, J=7.350, J=7.273), 1.229-1.275 (7-11, 17-22, 22H) 1.518-1.550 (12, 16, 23, 6H), 2.276-2.300 (5, 13, 24, 27, 7H), 2.469 (4, 1H, q, J=7.500) (Note: the spectrum was predicted in the website http://www.nmrdb.org/predictor).

Chapter 5: Olefin cross-metathesis, a mild, modular approach to functionalized cellulose esters

We include here FTIR and ^{1}H NMR spectra, and DSC data of new compounds prepared that we did not include in the body of the manuscript. All the results, unless otherwise stated below, were acquired using instruments and methods as described in the Experimental Section of the paper.

FTIR Spectra

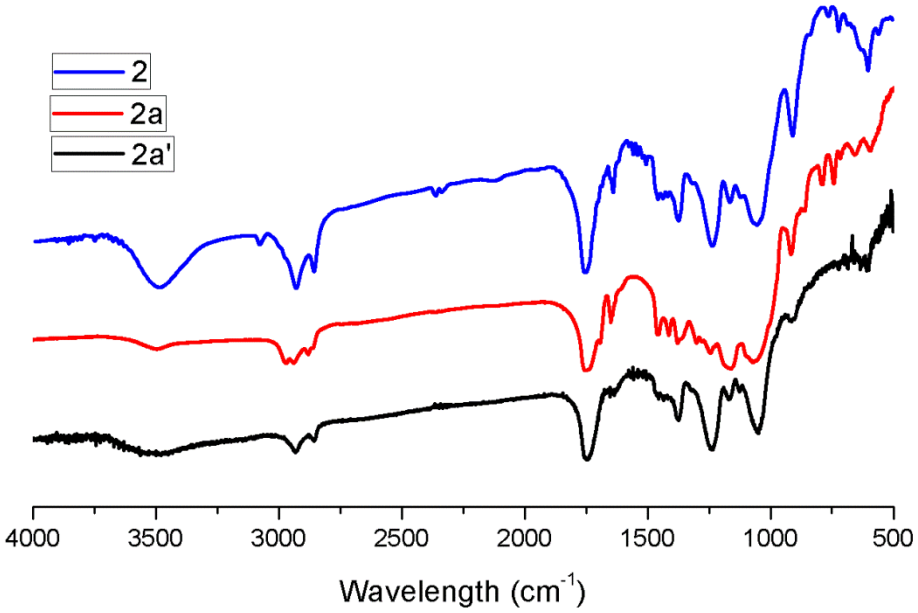


Fig. S5.1. FTIR spectra of terminally olefinic cellulose acetate undec-10-enoate **2**, the CM product (with AA) **2a**, and the hydrogenated product **2a'**.

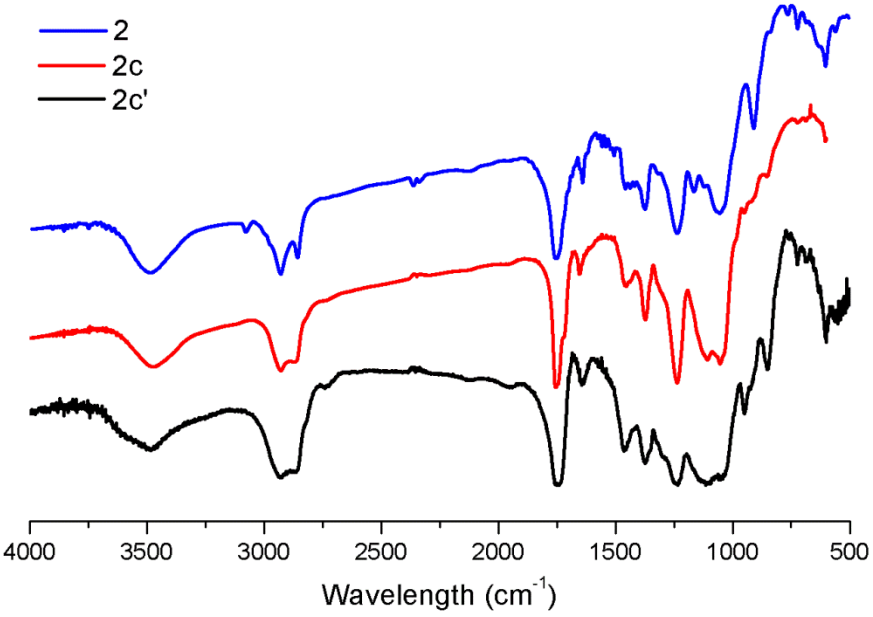


Fig. S5.2. FTIR spectra of terminally olefinic cellulose acetate undec-10-enoate **2**, the CM product (with PEGMEA) **2c**, and the hydrogenated product **2c'**.

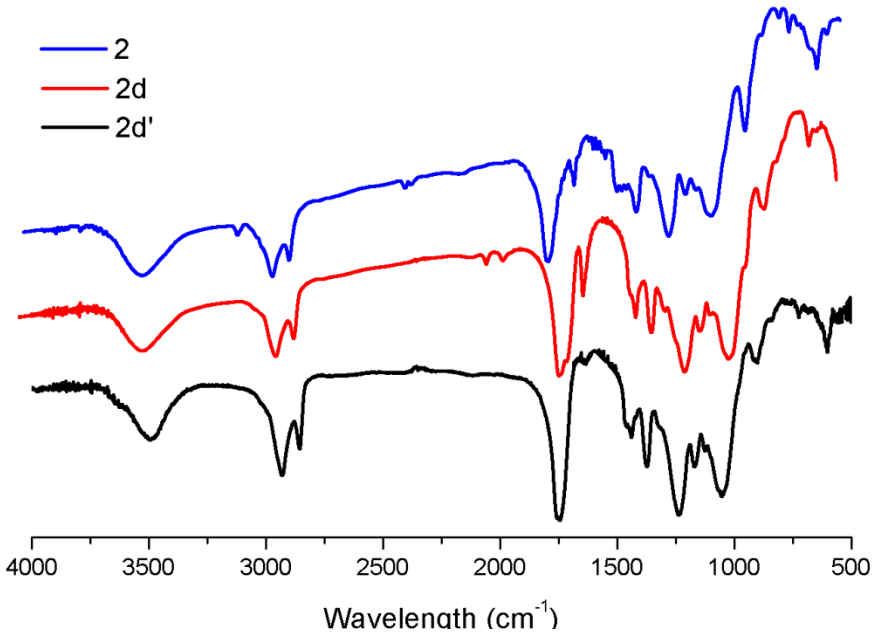


Fig. S5.3. FTIR spectra of terminally olefinic cellulose acetate undec-10-enoate **2**, the CM product (with MA) **2d**, and the hydrogenated product **2d'**.

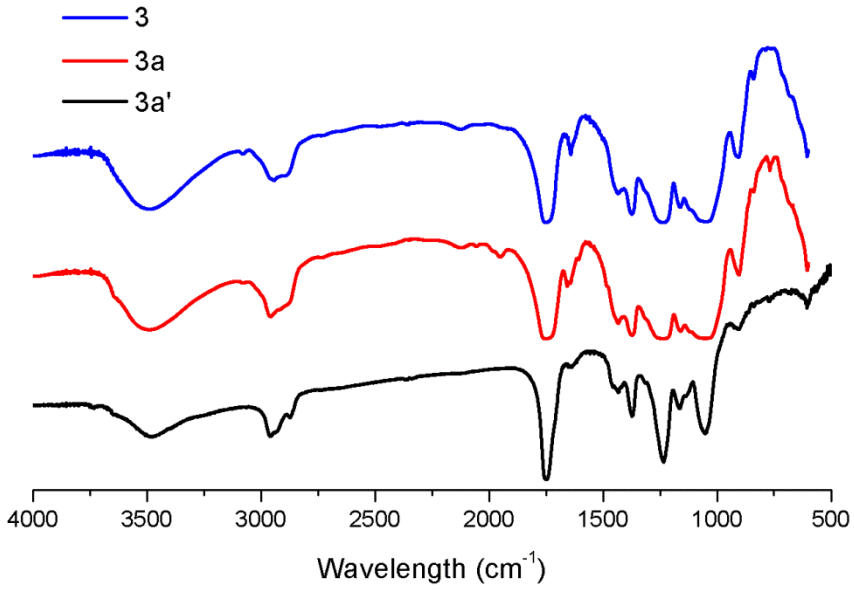


Fig. S5.4. FTIR spectra of terminally olefinic cellulose acetate pen-4-tenoate **3**, the CM product (with AA) **3a**, and the hydrogenated product **3a'**.

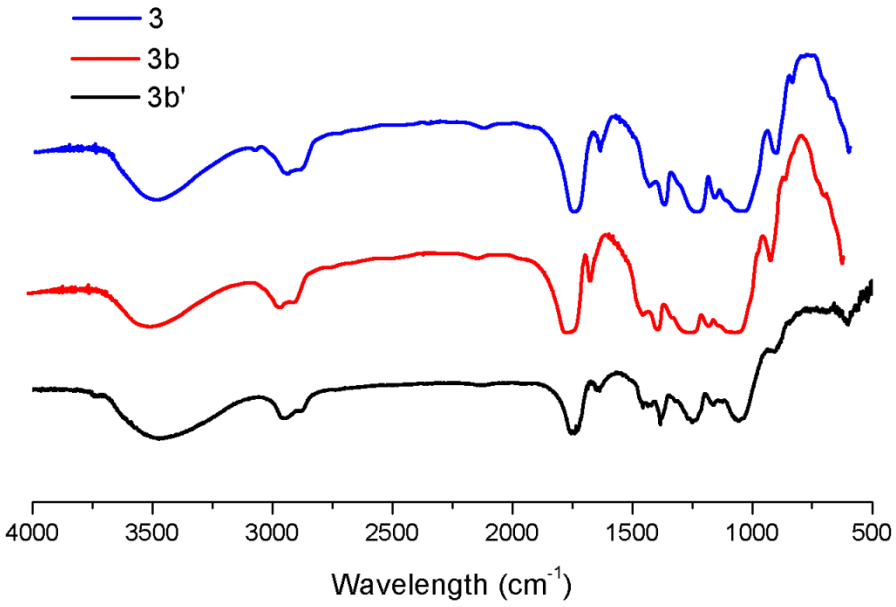


Fig. S5.5. FTIR spectra of terminally olefinic cellulose acetate pen-4-tenoate **3**, the CM product (with HEA) **3b**, and the hydrogenated product **3b'**.

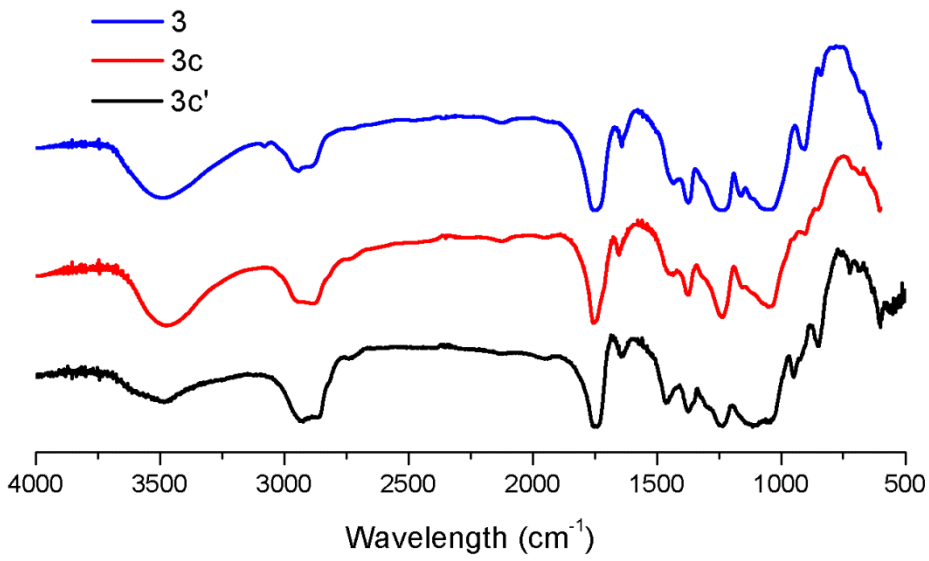


Fig. S5.6. FTIR spectra of terminally olefinic cellulose acetate pen-4-tenoate **3**, the CM product (with PEGMEA) **3c**, and the hydrogenated product **3c'**.

NMR spectra

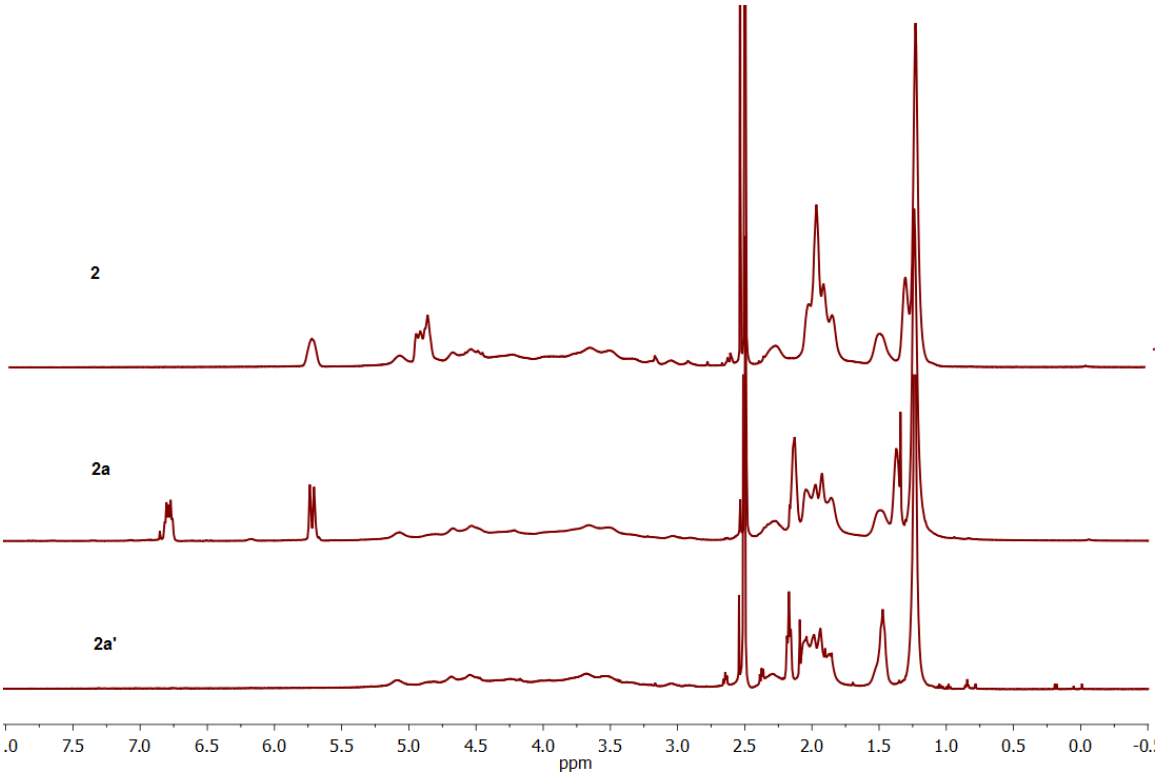


Fig. S5.7. ^{1}H NMR spectra of terminally olefinic cellulose acetate undec-10-enoate **2**, CM product (with AA) **2a**, and hydrogenated product **2a'**.

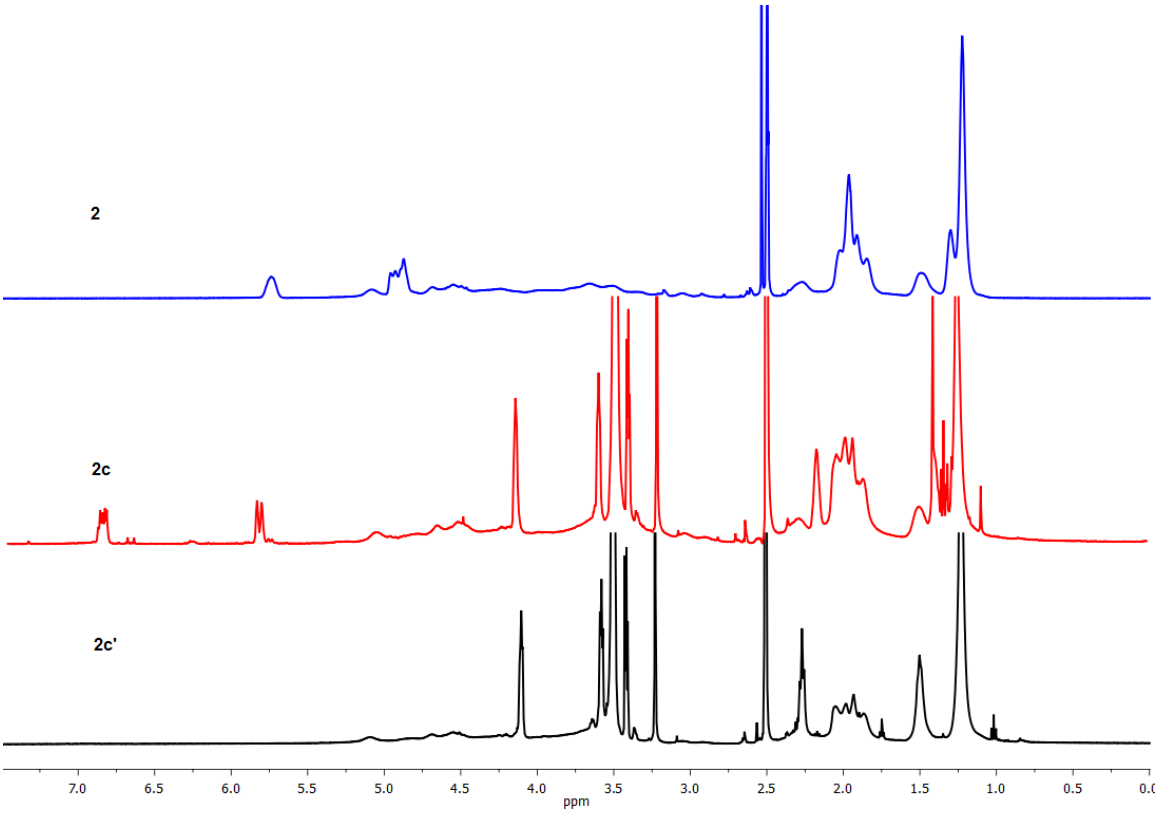


Fig. S5.8. ^{1}H NMR spectra of terminally olefinic cellulose acetate undec-10-enoate **2**, CM product (with PEGMEA) **2c**, and hydrogenated product **2c'**.

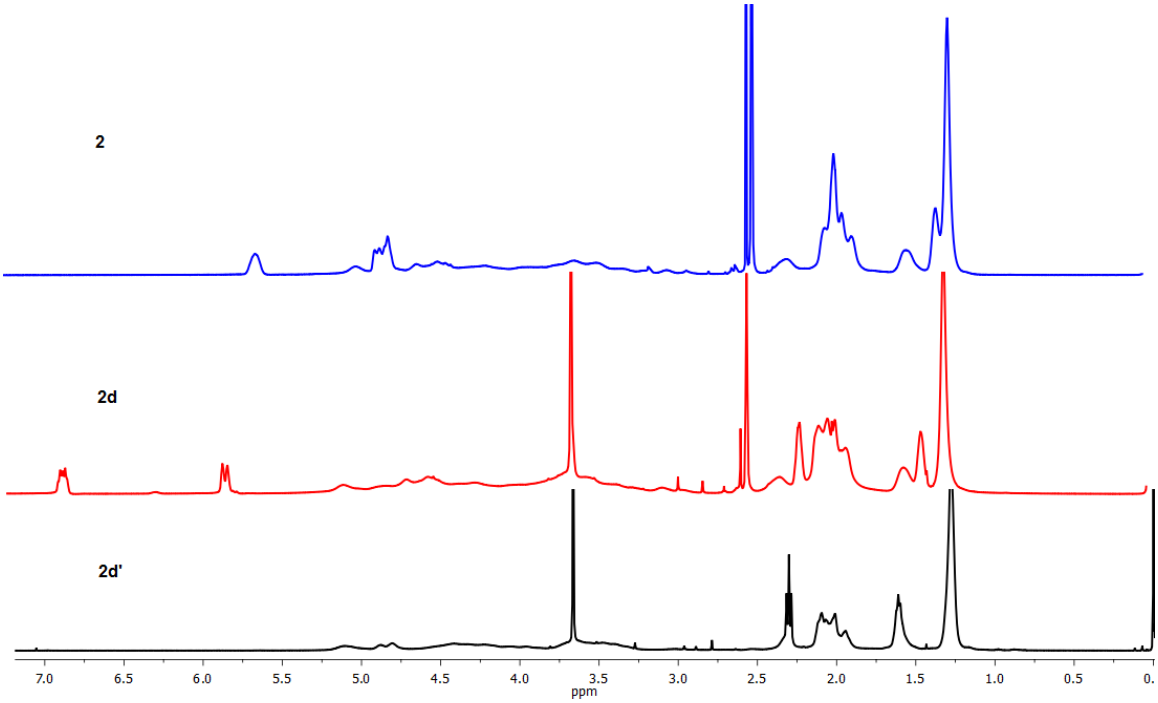


Fig. S5.9. ^{1}H NMR spectra of terminally olefinic cellulose acetate undec-10-enoate **2**, CM product (with MA) **2d**, and hydrogenated product **2d'**.

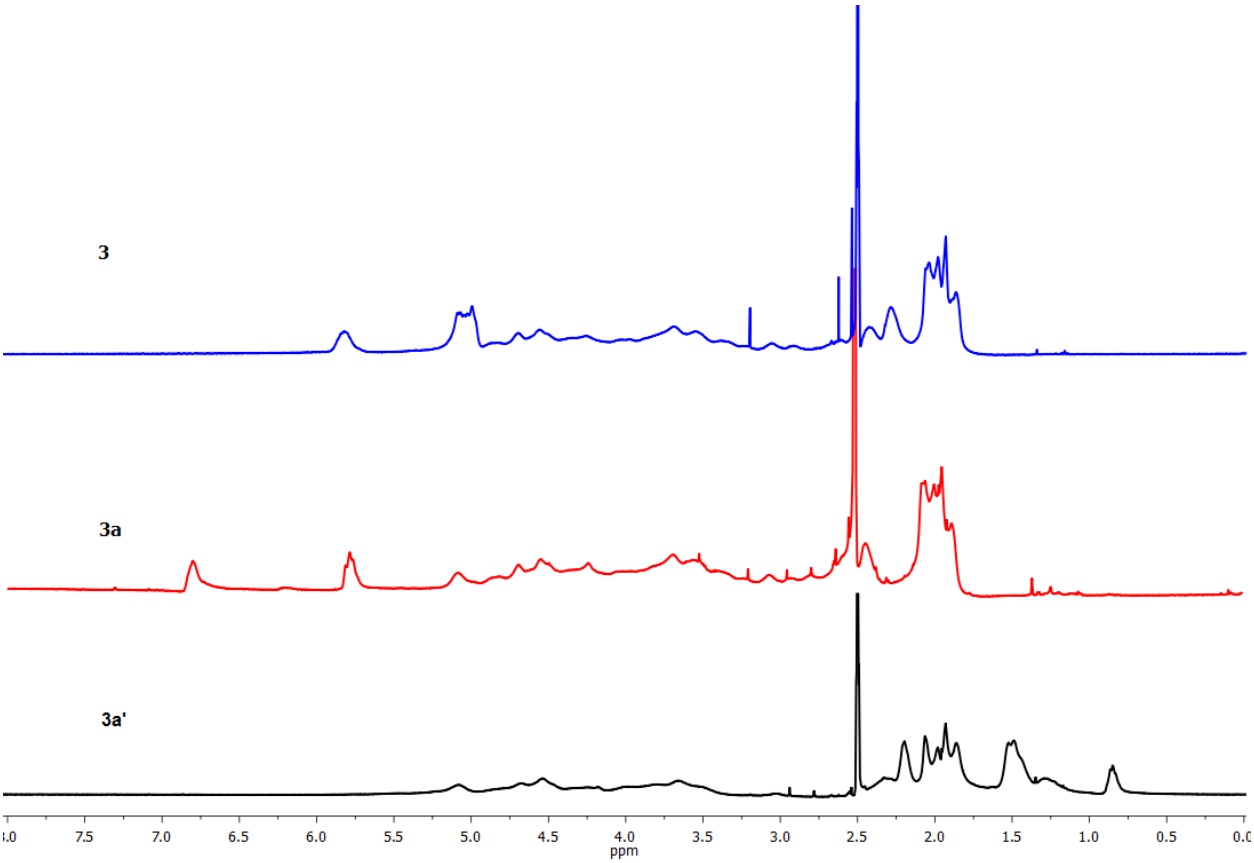


Fig. S5.10. ^{1}H NMR spectra of terminally olefinic cellulose acetate pent-4-enoate **3**, CM product (with AA) **3a**, and hydrogenated product **3a'**.

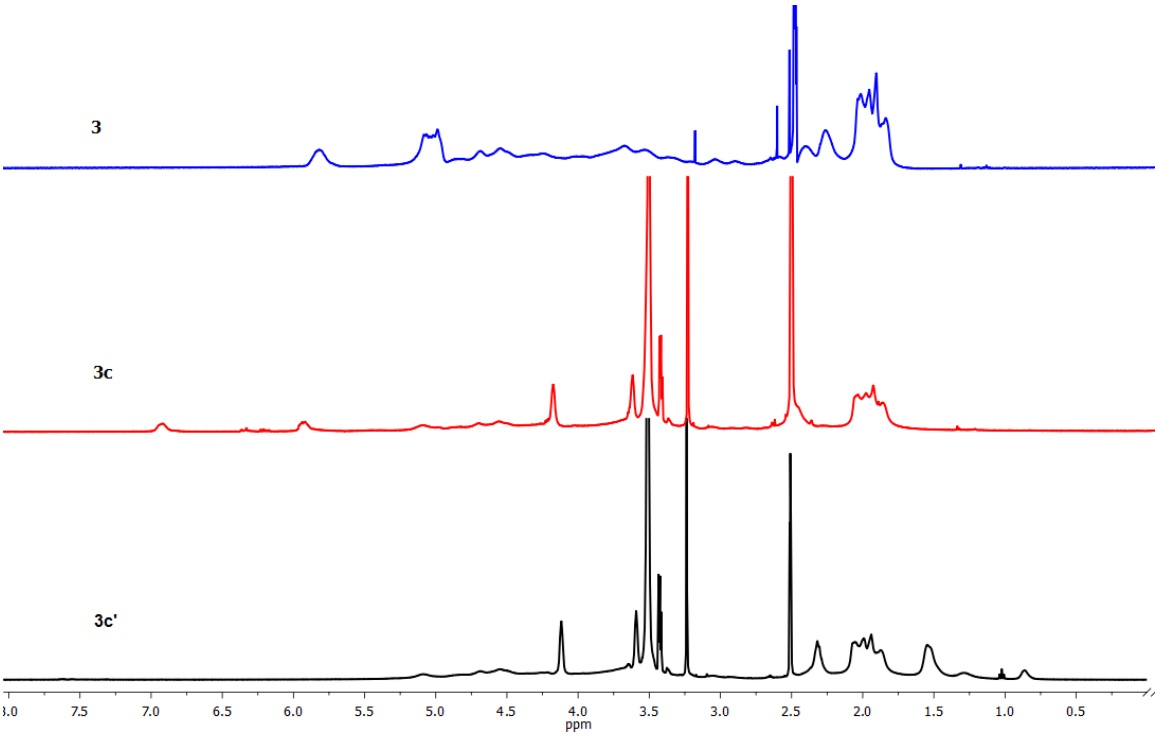


Fig. S5.11. ^{1}H NMR spectra of terminally olefinic cellulose acetate pent-4-enoate **3**, CM product (with PEGMEA) **3c**, and hydrogenated product **3c'**.

DSC data

Table S5.12. T_g of the CM products (2a-2d, 3a-3c) measured by DSC.

Compound	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**
T_g (ºC)	115	109	NA	100	151	137	78

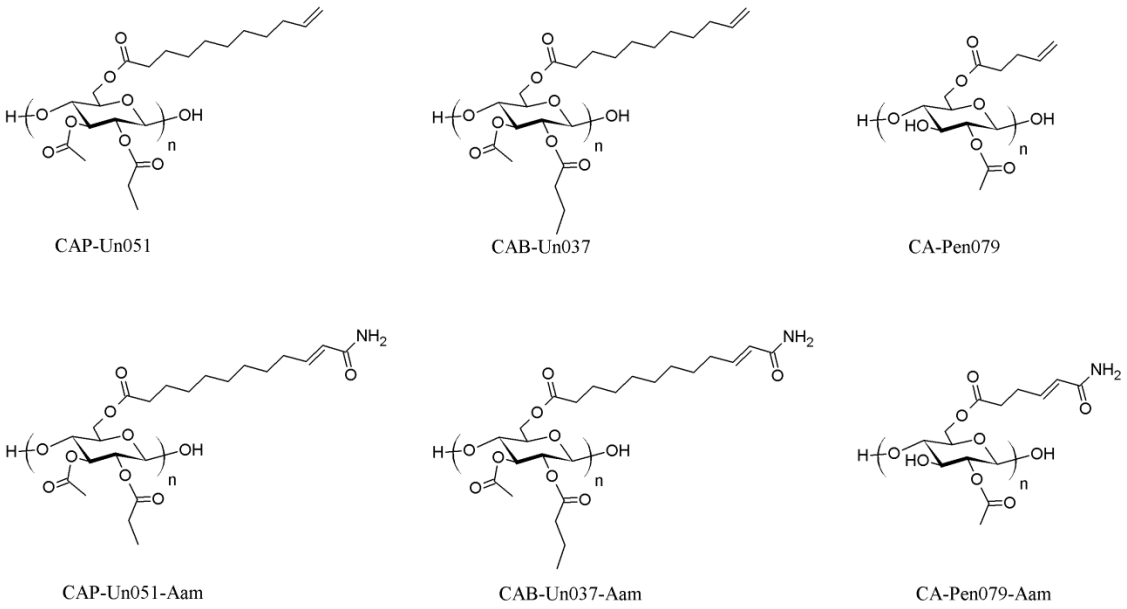


Fig. S6.1. Chemical structures of cellulose esters including cellulose acetate propionate undec-10-enoate (CAP-Un051), cellulose acetate butyrate undec-10-enoate (CAB-Un037), and cellulose acetate pent-enoate (CA-Pen-079), and the corresponding CM products (with acrylamide): CAP-Un-Aam, CAB-Un-Aam, and CA-Pen-Aam. (Note that structures are not meant to imply regiospecificity; particular positions of substitution in all schemes are only for convenience of depiction and clarity.)

The syntheses of CAP-Un051 and CAB-Un037 were according to our previous published procedures[1]. In detail, CAP-504-0.2 (1.00 g, 1.78 mmol/AGU) was dissolved in MEK (20 mL), and the solution was heated to 60 °C with magnetic stirring under N_2. After the addition of triethylamine (0.54 mL, 1.96 mmol, 1.1 equiv), 10-undecenoyl chloride (0.72 g, 3.56 mmol, 1.0 equiv) was added dropwise, and the mixture was stirred for 20 h at 60 °C. After filtration to remove triethylammonium chloride, the filtrate was precipitated into 300 mL of 50:50 water/ethyl alcohol. The product (CAP-Un051) was redissolved in CH_2Cl_2, reprecipitated in hexane, and dried under vacuum at 40 °C. A similar procedure was followed for the preparation of CAB-Un037.

The synthesis of CA-Pen079 was modified from our previous published method. CA-320S (1.00 g, 4.19 mmol/AGU) was dissolved in DMI (20 mL), and the solution was heated to

90 °C with mechanical stirring under N_2. Triethylamine (3.3 mL, 2.2 equiv.) was added; a condenser was used to avoid evaporative loss of the base catalyst. 4-Pentenoyl chloride (2.49 g, 10.47 mmol, 2.5 equiv.) was added dropwise and allowed to react at 90 °C for 20 h. The reaction mixture was then filtered, and the filtrate was precipitated in 300 mL 50 : 50 water–ethyl alcohol. The precipitate was redissolved in a minimal amount of CH2Cl2 and reprecipitated in hexane. The product was washed with hexane and dried under vacuum at 40 °C.

The CM reactions toward CAP-Un051-Aam, CAB-Un037-Aam, and CA-Pen079-Aam were performed in acetic acid using the same procedure as described in the Experimental Section of manuscript main body.

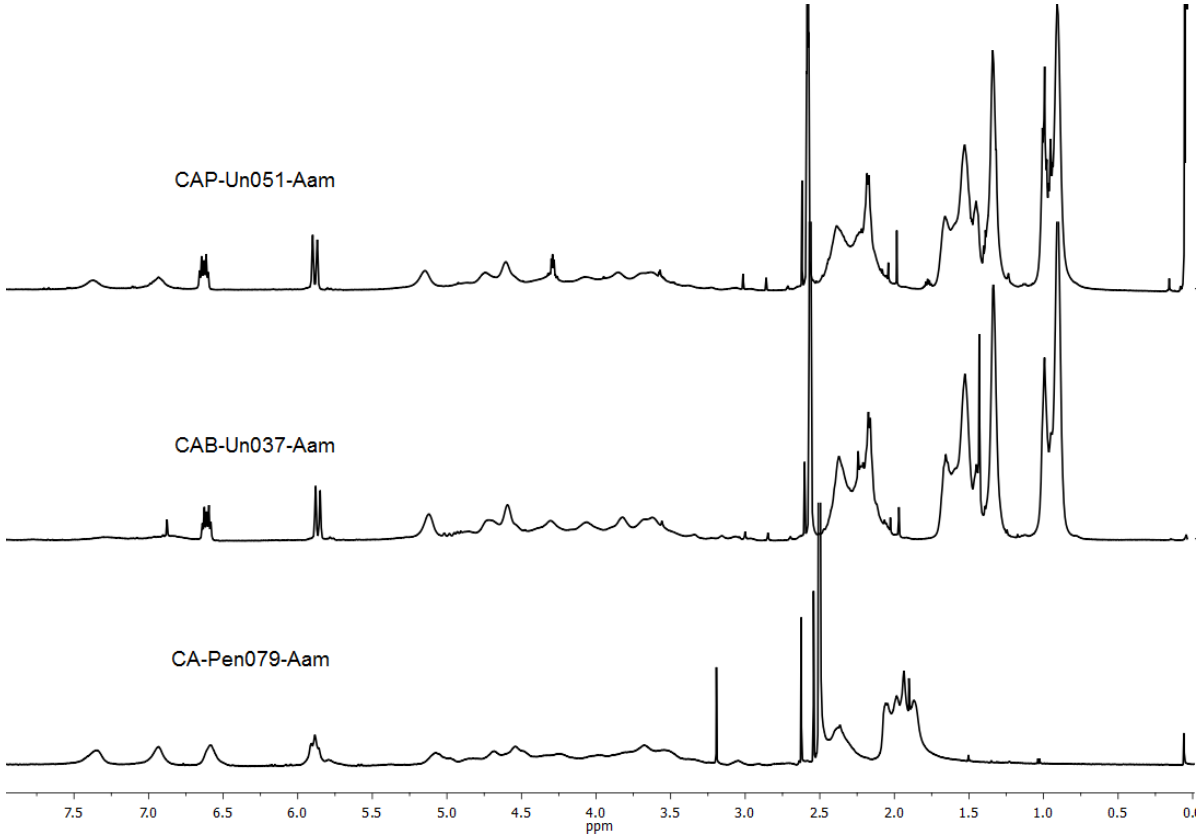
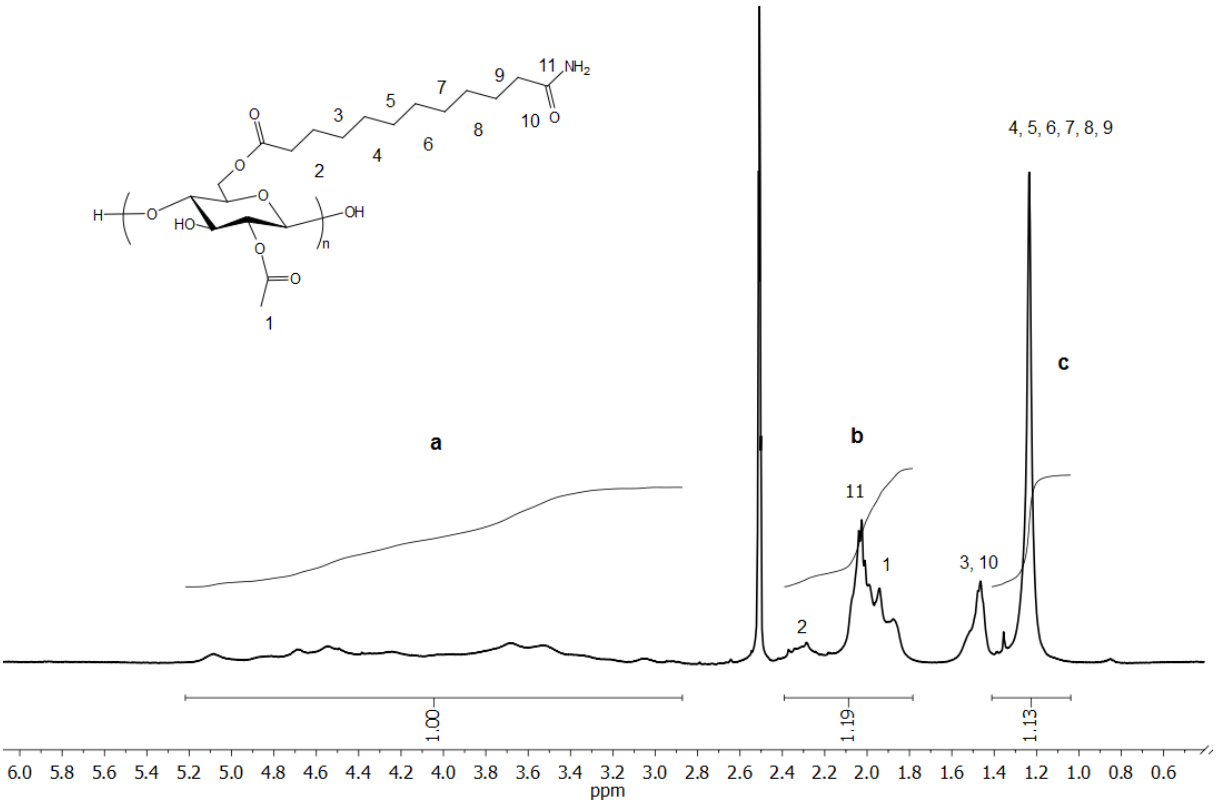


Fig. S6.2. NMR spectra of CAP-Un051-Aam, CAB-Un037-Aam, and CA-Pen079-Aam.

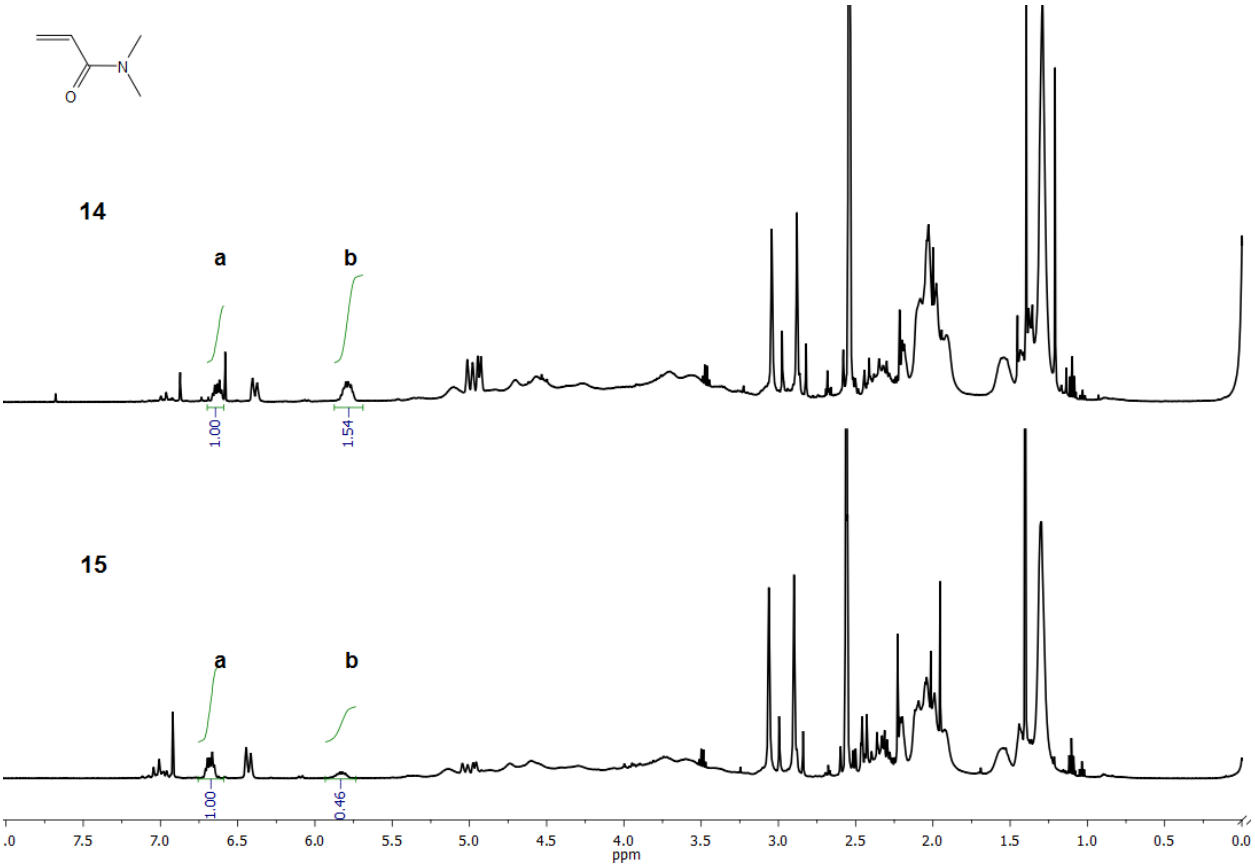


DS(amide) and DS(Ac) were calculated from equations:

$$\frac{I(b)}{I(a)} = \frac{2\times2\times DS(amide) + 3\times DS(Ac)}{7}$$

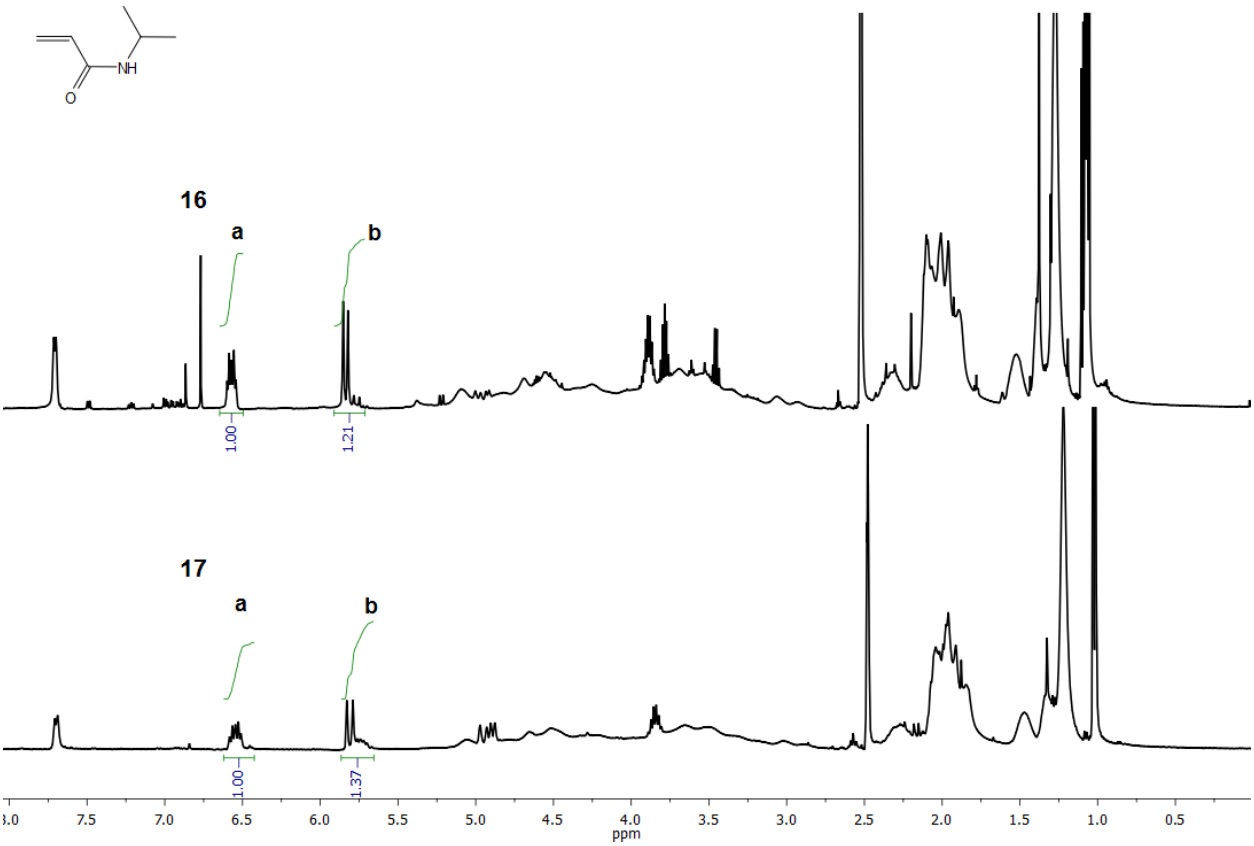
$$\frac{I(c)}{I(a)} = \frac{6\times2\times DS(amide)}{7}$$

Fig. S6.3. ^{1}H NMR spectrum of CA-Un067-Aam-H.



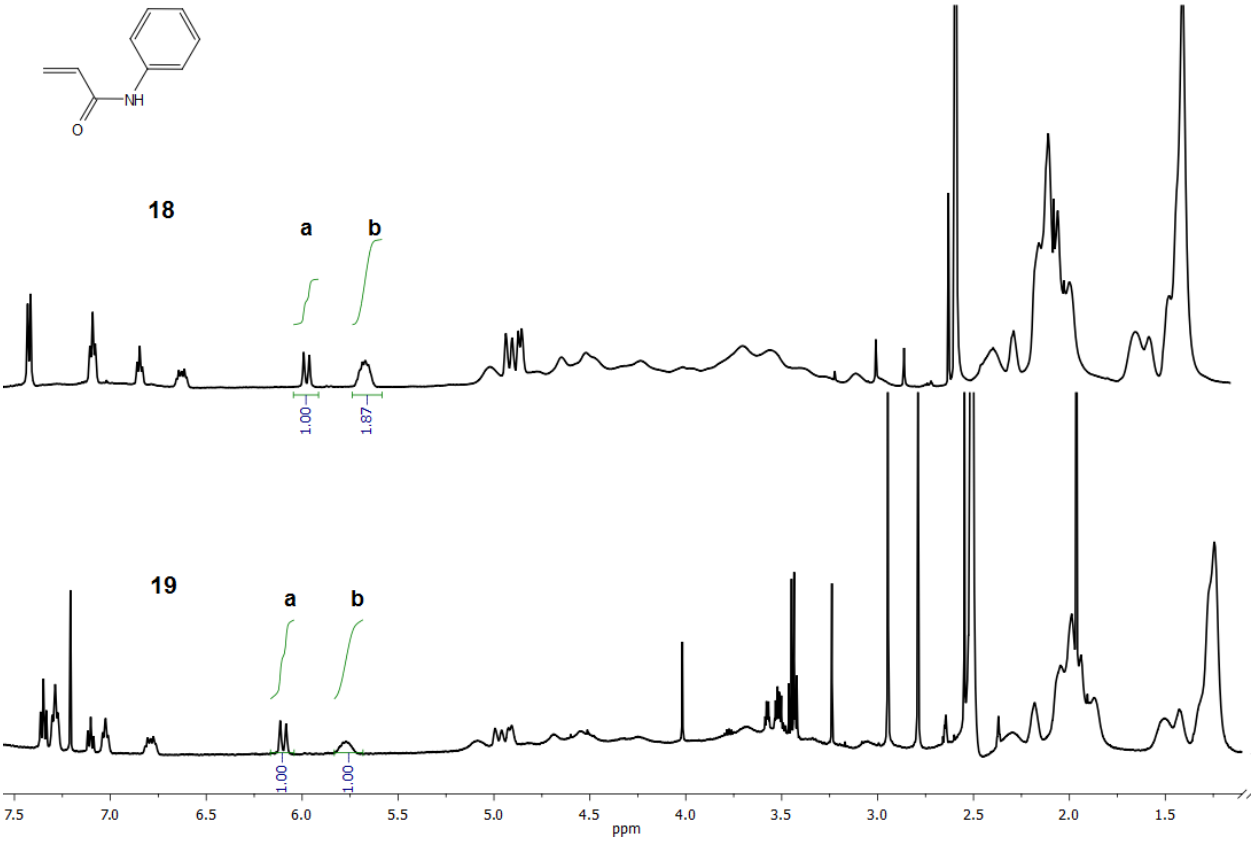
$$Conversion = \frac{I(a)}{I(a) + I(b)} \times 100\%$$

Fig. S6.4. [1]H NMR spectra of polymer 14 and 15 from Table 3.



$$Conversion = \frac{I(a)}{I(b)} \times 100\%$$

Fig. S6.5. 1H NMR spectra of polymer 16 and 17 in Table 3.



$$Conversion = \frac{I(a)}{I(a) + I(b)} \times 100\%$$

Fig. S6.6. ^{1}H NMR spectra of polymer 18 and 19 from Table 3.

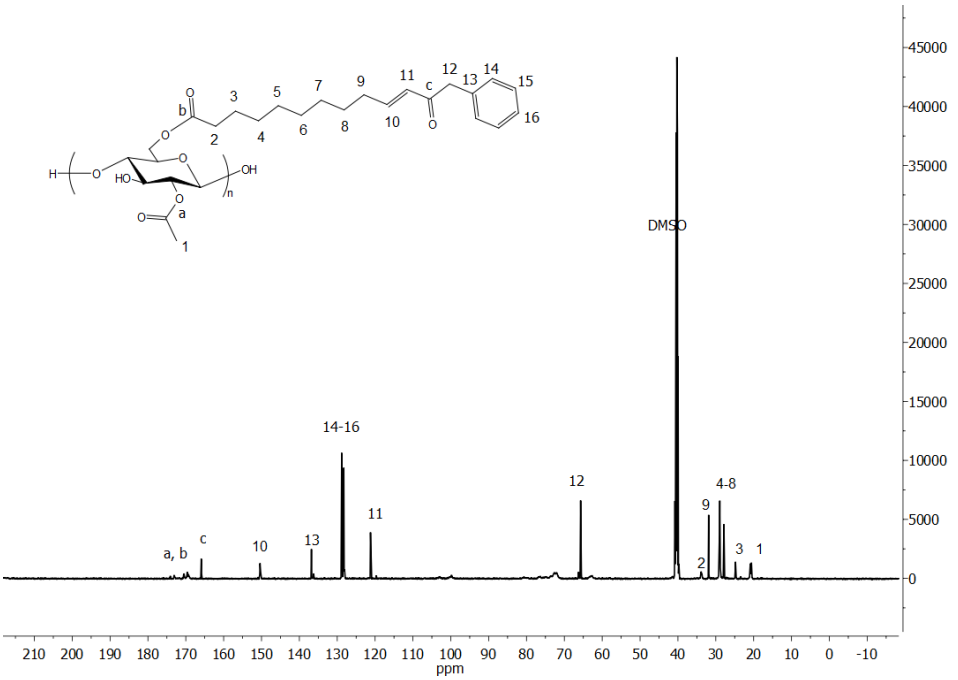


Fig. S7.1. Carbon NMR spectrum of polymer **1** CA-Un067-BA.

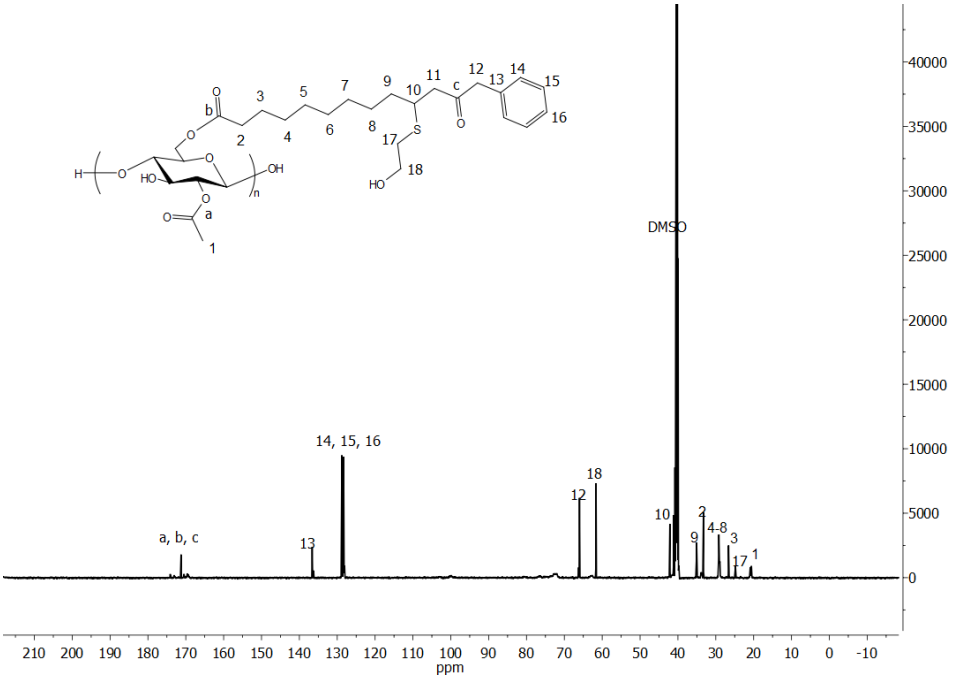


Fig. S7.2. Carbon NMR spectrum of polymer **7** CA-Un067-BA-ME

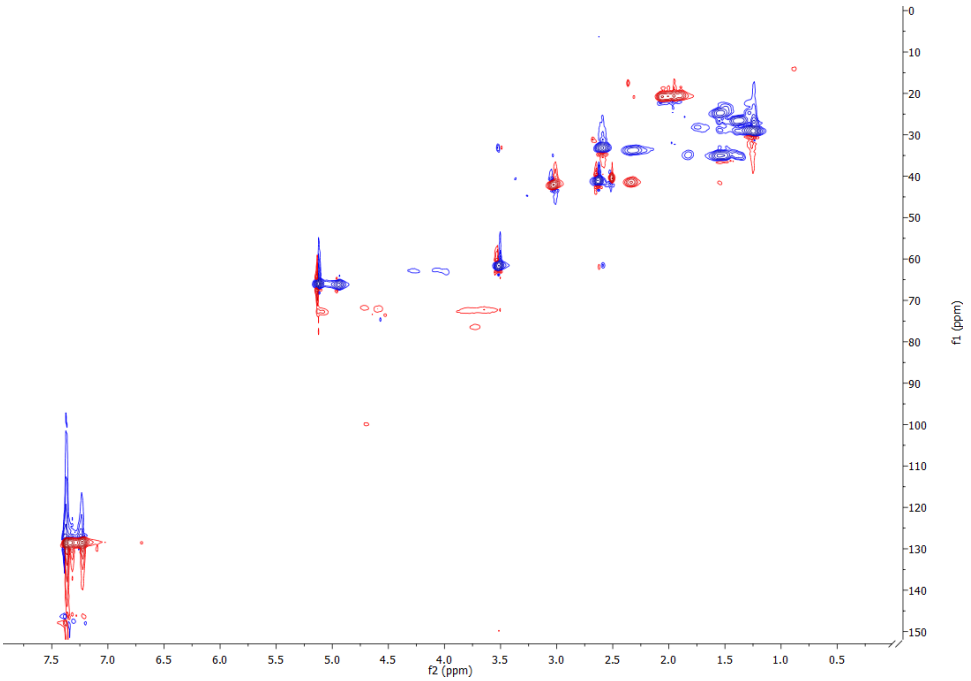


Fig. S7.3. 1H-13C HSQC spectrum of polymer 7 CA-Un067-BA-ME.

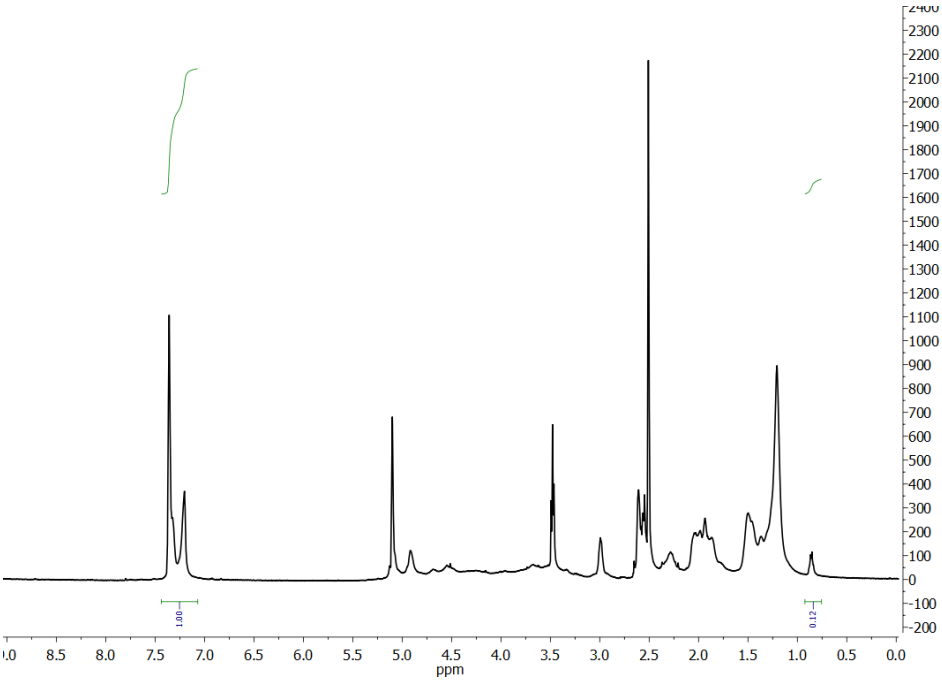


Fig. S7.4. Proton NMR spectrum of CA-Un067-BA-ME catalyzed by high amount of hexylamine (olefin/2-ME/HA ratio = 1/6/6).

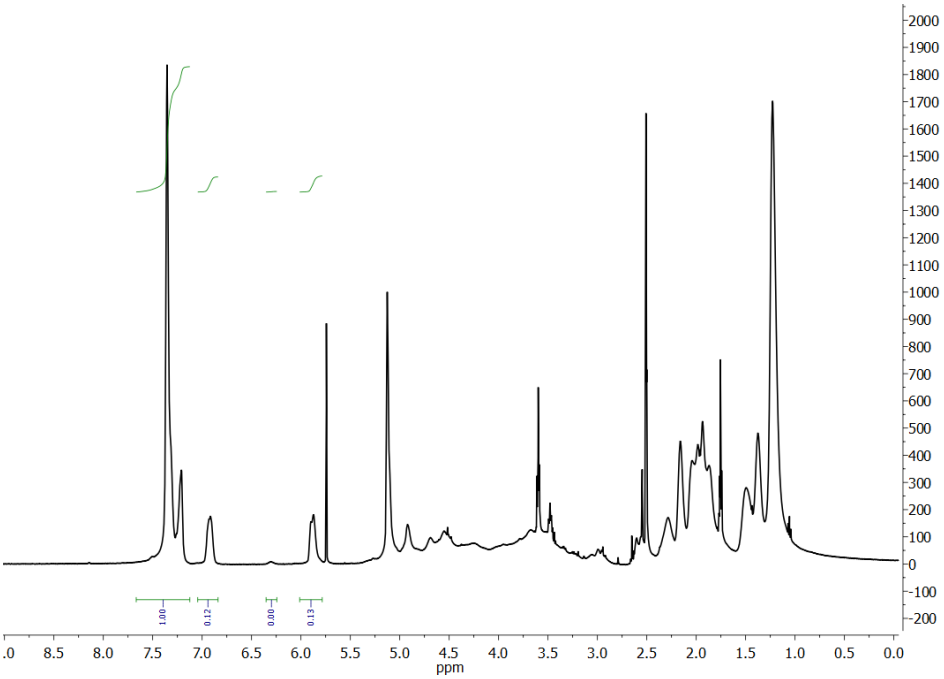


Fig. S7.5. Proton NMR of polymer 6 CA-Un067-BA-ME catalyzed by TEA. Calculated from integrations of NMR peaks, the conversion of the reaction is 35%.

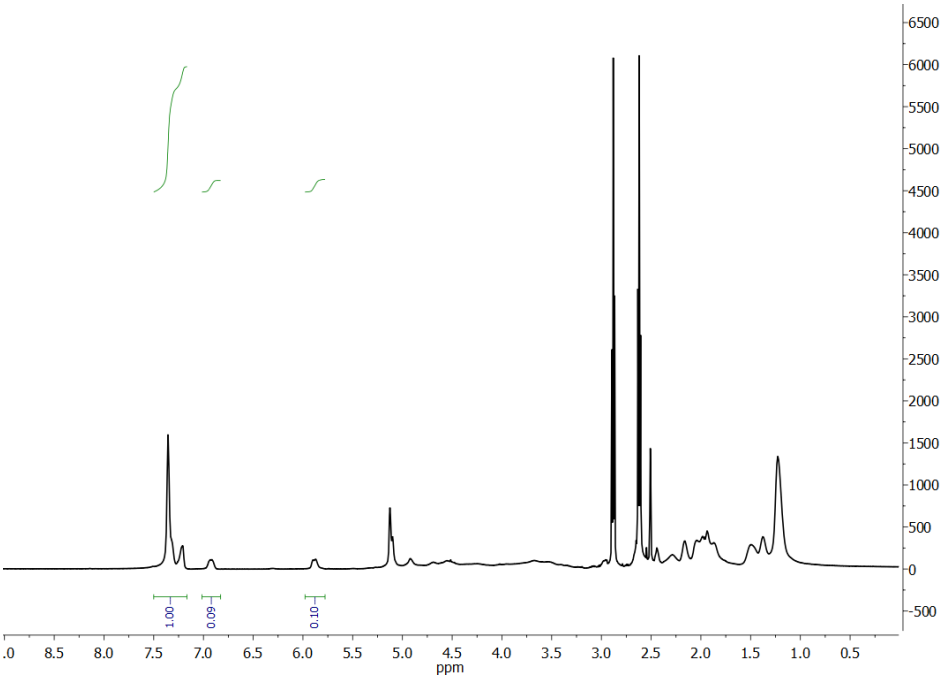


Fig. S7.6. Proton NMR spectrum of polymer 8 CA-Un067-BA-3MPA catalyzed by TEA. Calculated from integrations of NMR peaks, the conversion of the reaction is 50%.

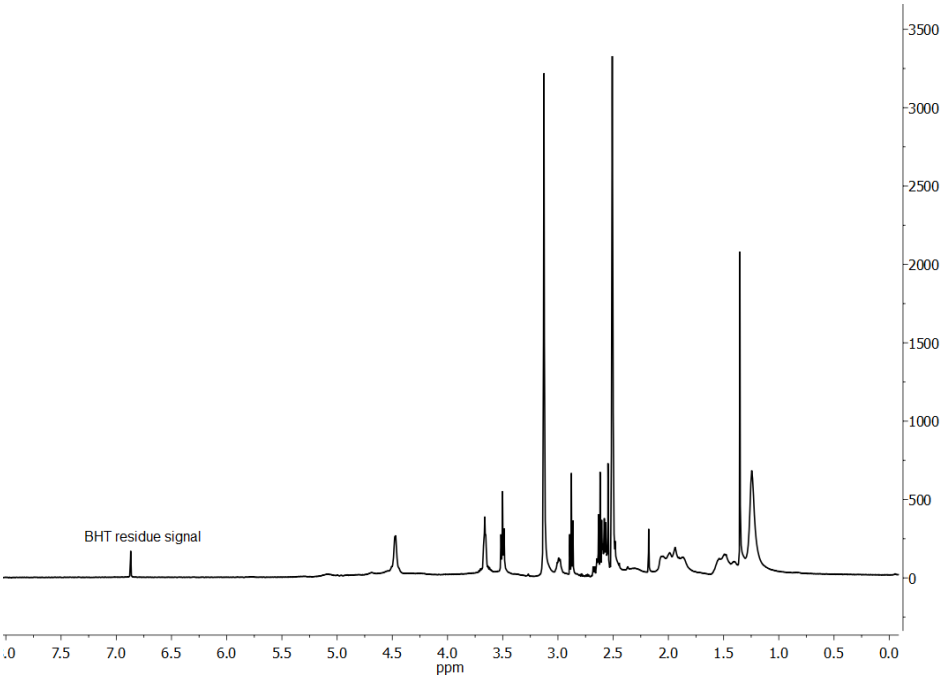


Fig. S7.7. Proton NMR spectrum of polymer 12 CA-Un067-TMA-2ME.

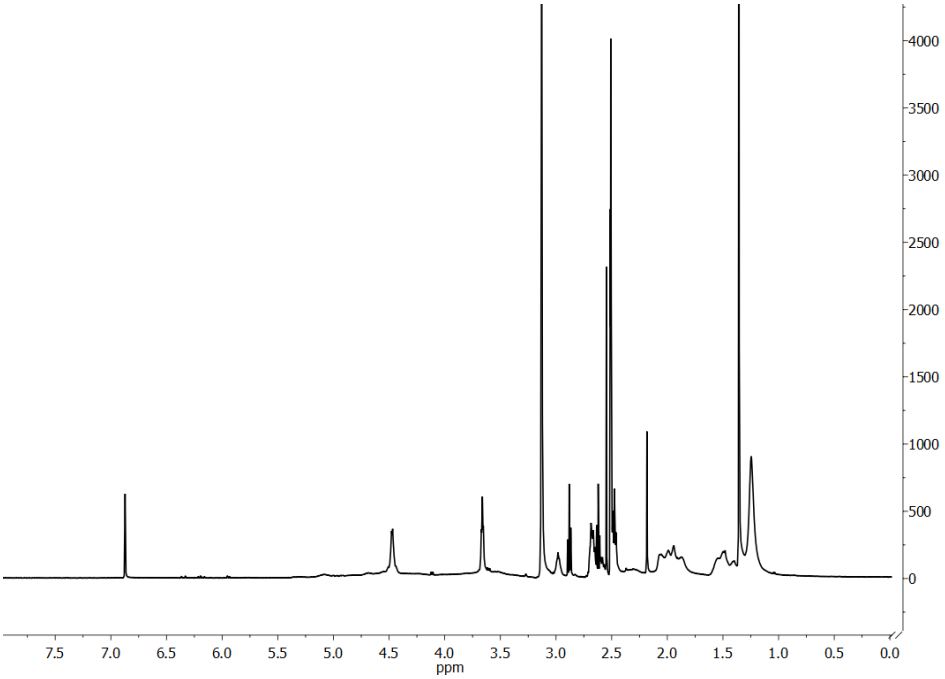


Fig. S7.8. Proton NMR spectrum of polymer 13 CA-Un067-TMA-3MPA.

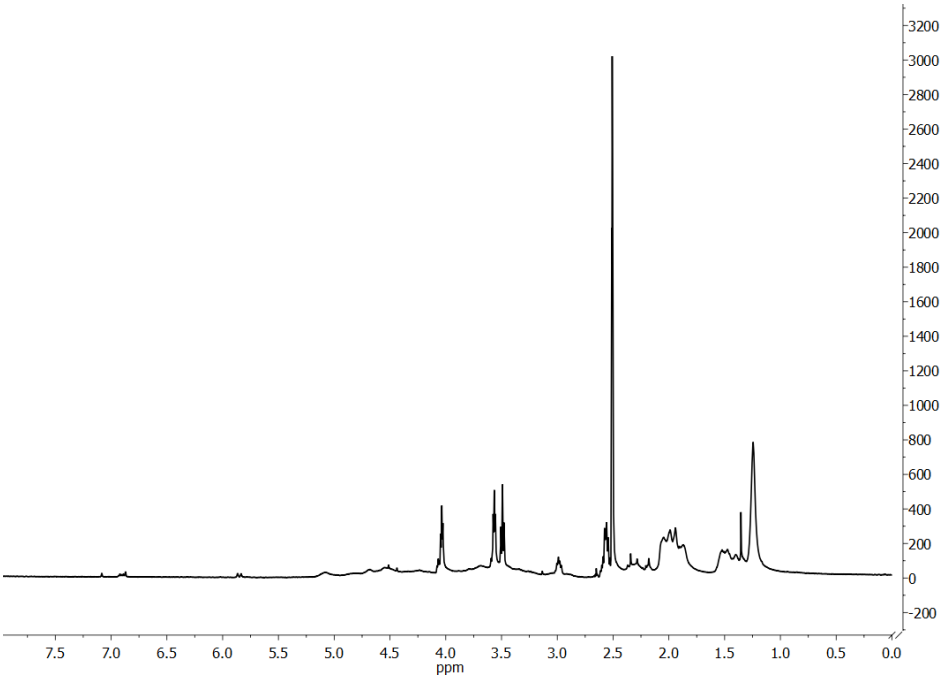


Fig. S7.9. Proton NMR spectrum of polymer 14 CA-Un067-HEA-2ME using TEA as catalyst.

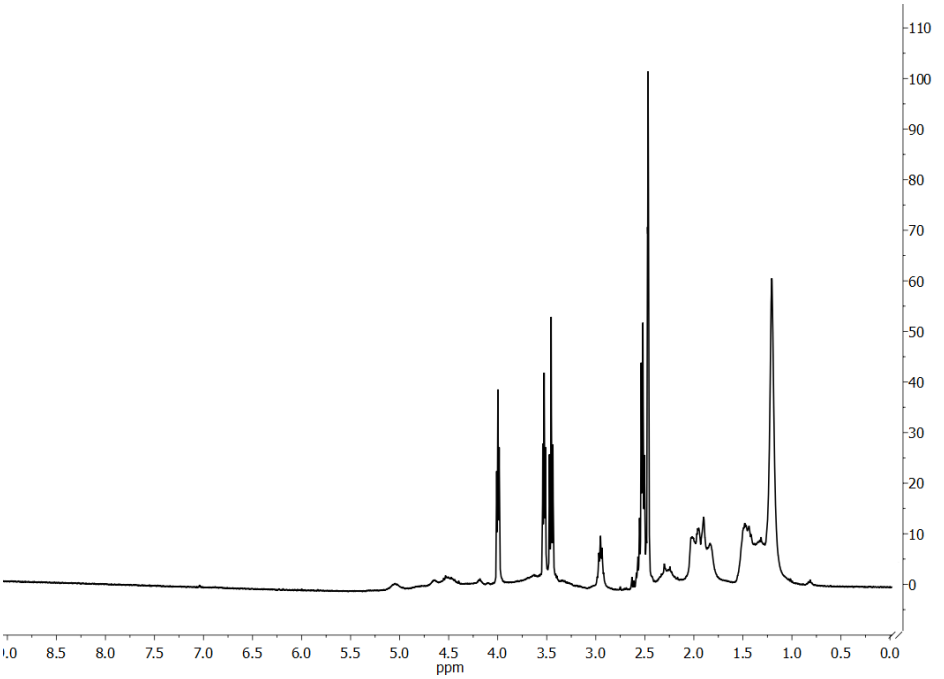


Fig. S7.10. Proton NMR spectrum of polymer 15 CA-Un067-HEA-2ME using HA as catalyst.

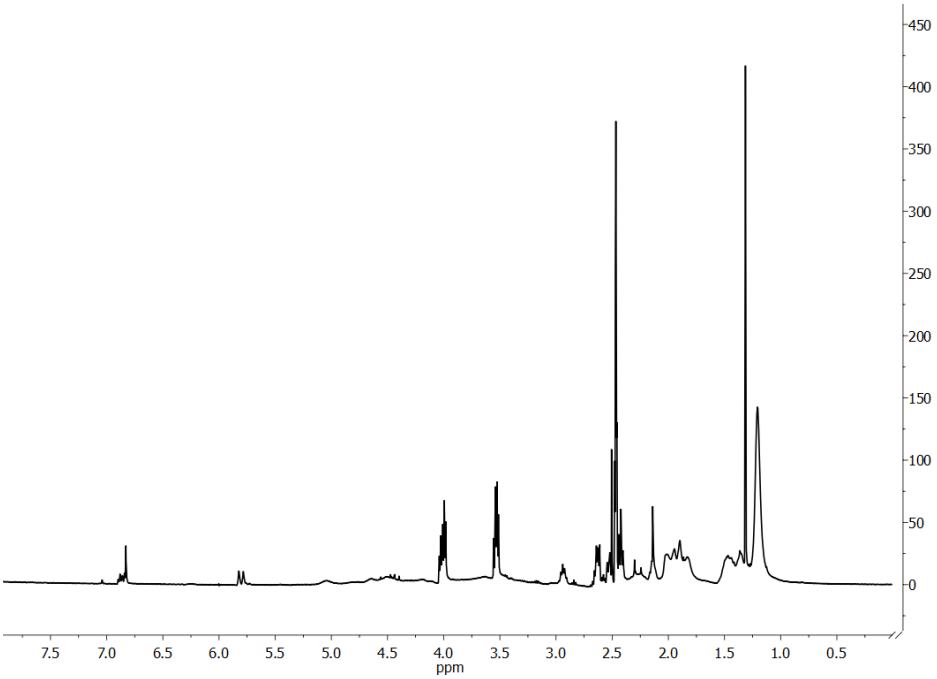


Fig. S7.11. Proton NMR spectrum of polymer 16 CA-Un067-HEA-3MPA using TEA as catalyst.

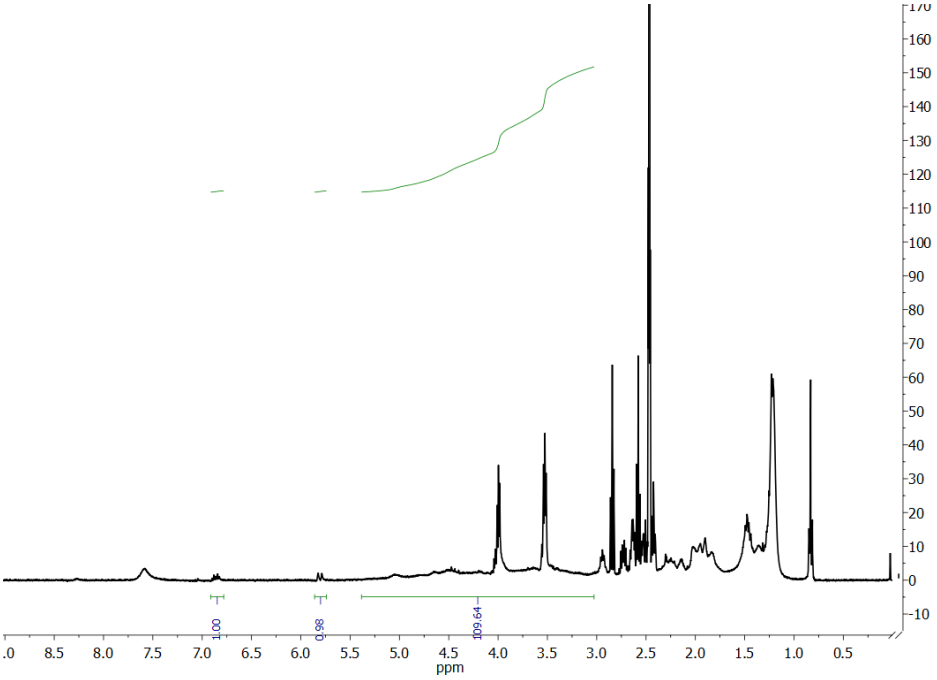


Fig. S7.12. Proton NMR spectrum of polymer 17 CA-Un067-HEA-3MPA using HA as catalyst.

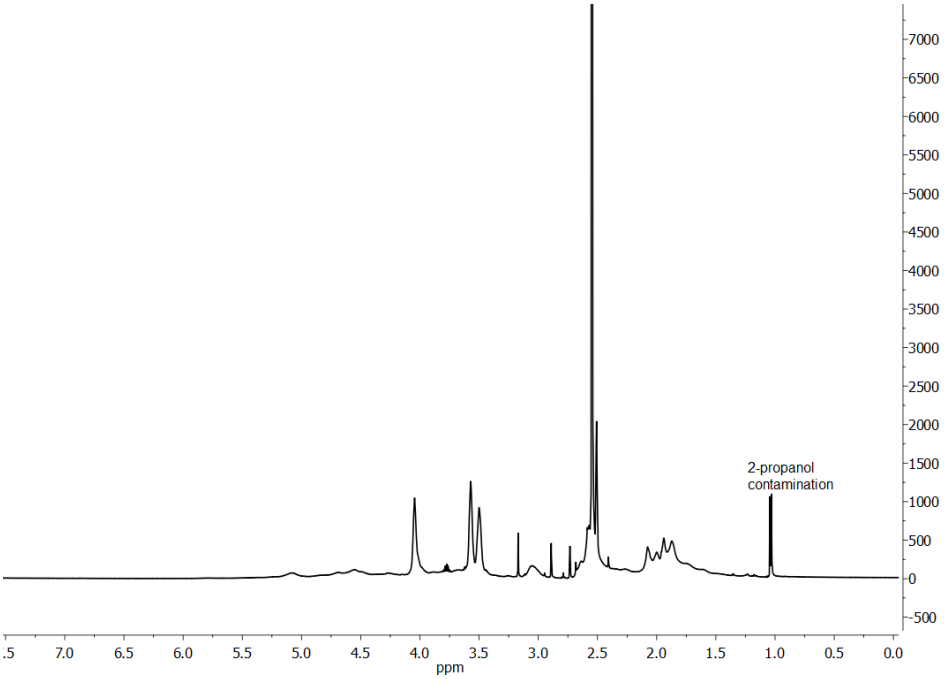


Fig. S7.13. Proton NMR spectrum of polymer 18 CA-Pen079-HEA-2ME using TEA as catalyst.

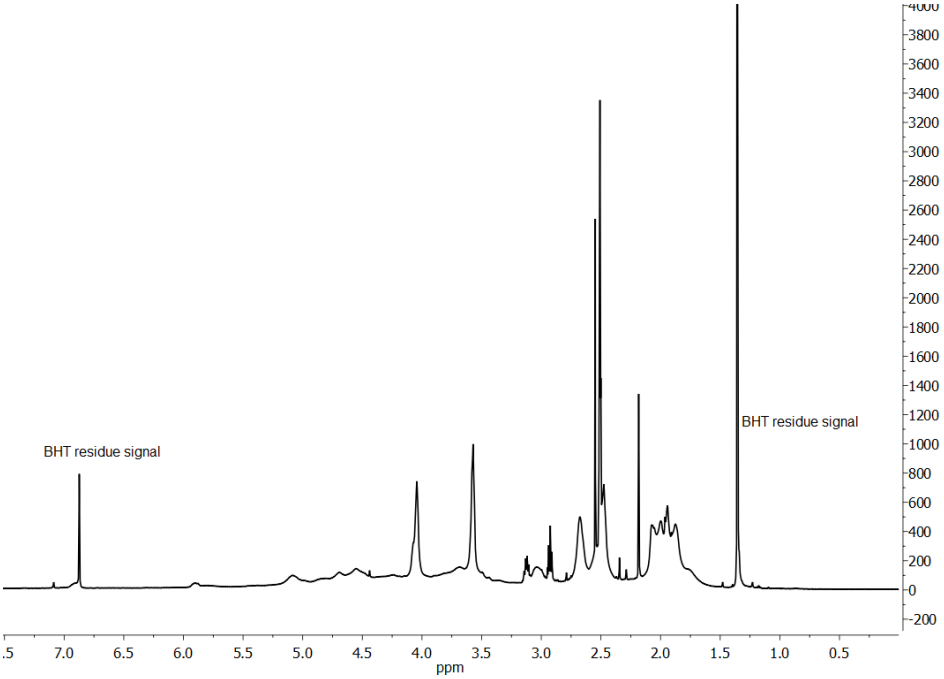


Fig. S7.14. Proton NMR spectrum of polymer 19 CA-Pen079-HEA-3MPA using TEA as catalyst.

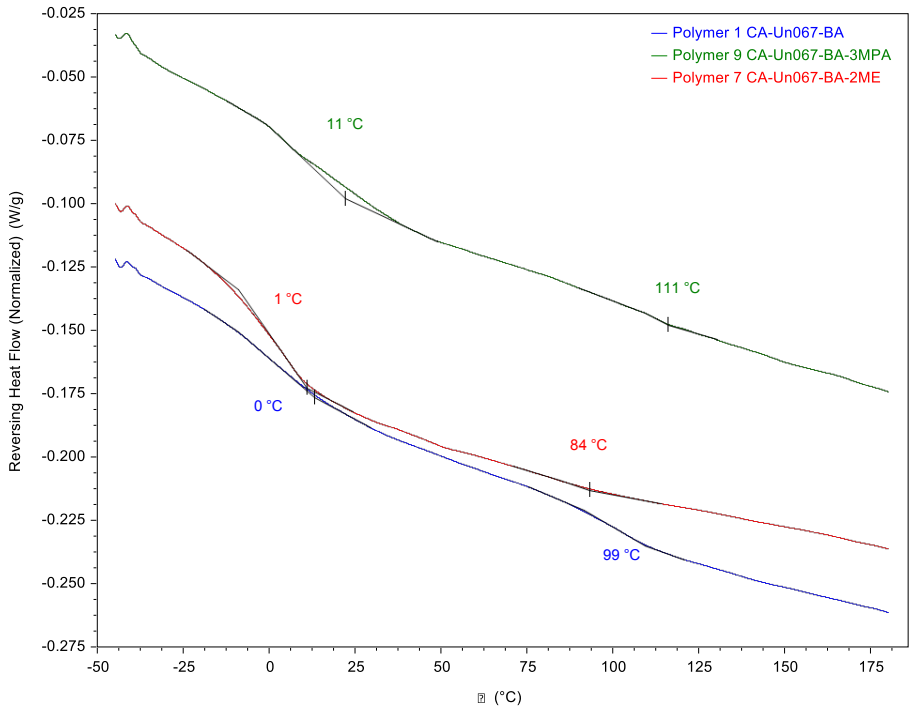


Fig. S7.15. DSC traces of polymers 1, 7 and 9.

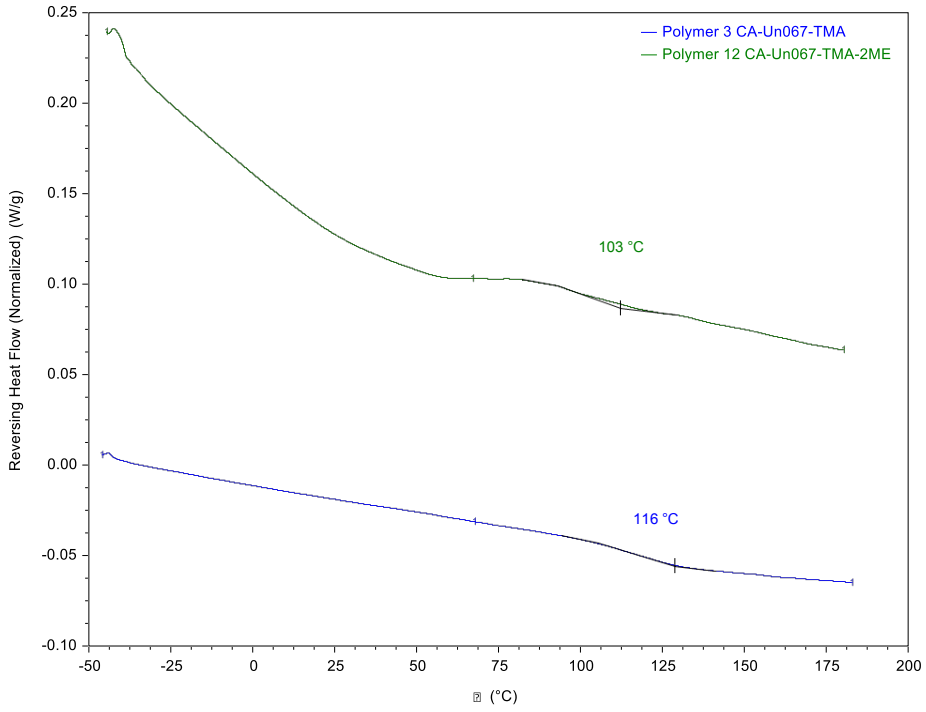


Fig. S7.16. DSC traces of polymers 3 and 12.

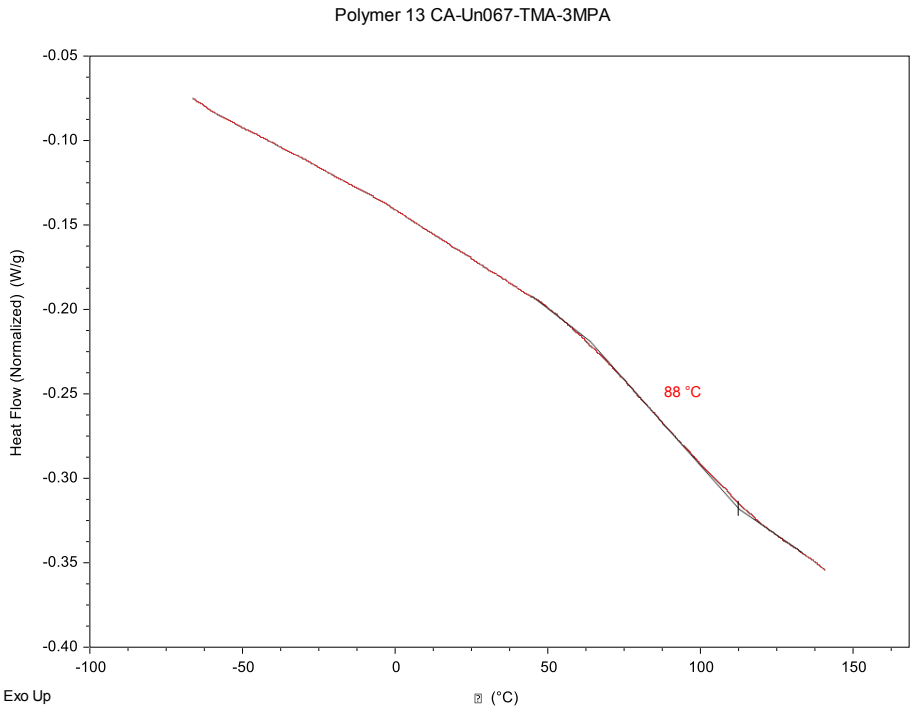


Fig. S7.17. DSC trace of polymer 13.

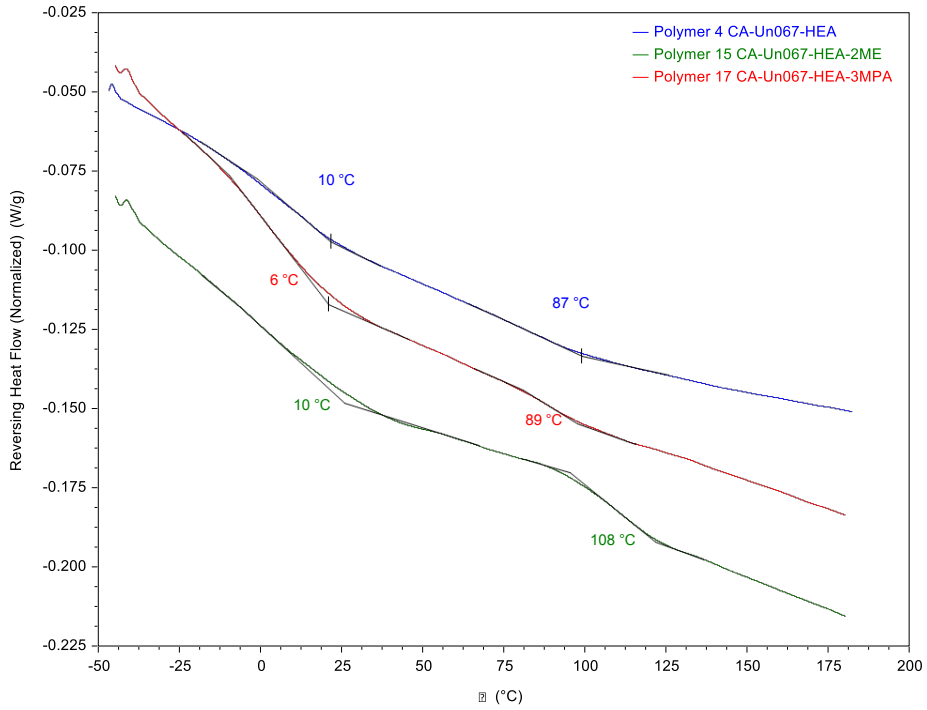


Fig. S7.18. DSC traces of polymers 4, 15 and 17.

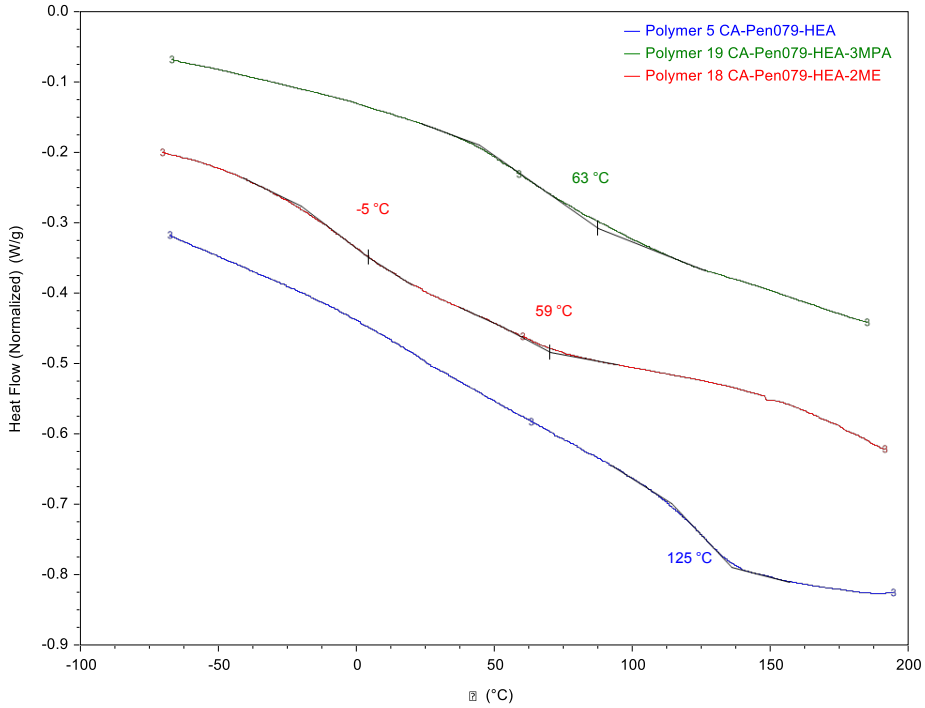


Fig. S7.19. DSC traces of polymers 5, 18 and 19.

(1) Meng, X.; Matson, J. B.; Edgar, K. J. *Biomacromolecules* **2014**, *15*, 177.